STUDY OF ANTI-CYSTICERCAL ANTIBODIES TO *TAENIA SOLIUM* IN THE SERUM OF EPILEPSY PATIENTS IN A TERTIARY CARE HOSPITAL

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CERTIFICATE

This is to certify that this dissertation entitled **"STUDY OF ANTI-CYSTICERCAL ANTIBODIES TO *TAENIA SOLIUM* IN THE SERUM OF EPILEPTIC PATIENTS IN TERTIARY CARE HOSPITAL"** is the bonafide original work done by **Dr.ARTHI E,** Post graduate in Microbiology, under my overall supervision and guidance in the department of Microbiology, Stanley Medical College, Chennai, in partial fulfillment of the regulations of The Tamil Nadu Dr. M.G.R. Medical University for the award of **M.D Degree in Microbiology (Branch IV).**

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DECLARATION

I solemnly declare that this dissertation **"STUDY OF ANTI-CYSTICERCAL ANTIBODIES TO *TAENIA SOLIUM* IN THE SERUM OF EPILEPTIC PATIENTS IN TERTIARY CARE HOSPITAL"**is the bonafide work done by me at the Department of Microbiology, Government Stanley Medical College Hospital, Chennai, under the guidance and supervision of **Prof. Dr.R.SELVI, M.D.,** Professor and head of department of Microbiology, Government Stanley Medical College, Chennai-600 001.

This dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the University regulations for the award of degree of M.D. Branch IV Microbiology examinations to be held in April 2013.

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CONTENTS

INTRODUCTION

Cysticercosis is a parasitic infection of the larval form of the zoonotic cestode , *Taenia solium*. When humans are infected with the adult worm or the larval stage it causes Taenisis and Cysticercosis respectively. Infection of the central nervous system with the larval form is called Neurocysticercosis (NCC). It is the most common parasitic infection of the central nervous system[27,28]. It is also the leading cause of epilepsy in the developing world [3,29,60] .Neurocysticercosis is a disease associated with poverty and poor hygienic practices . It is the disease of major public health importance in the developing countries especially Latin America, India, Africa and China [64]. Recently, there has been rising trends of the disease in the developed countries due to ease of international travel and increasing immigrants from the endemic areas [60]. About 2.5 million people worldwide are tapeworm carriers and nearly 20 million people are infected with cysticerci [38] . Cysticercosis / Taeniasis is emerging as a serious public health and agricultural problem in many developing areas of the world.

Neurocysticercosis presents with varied clinical manifestation. Epileptic seizure is the most common presentation seen in 70-90% of the

cases [76, 16]. NCC is the most common cause of late onset epilepsy in areas of endemicity[41] . Epilepsy due to NCC is a major problem in tropical developing countries. A recent meta-analysis of published studies has estimated that about 29.6% (95%CI: 23.5%-36.1%) of epilepsy cases in the NCC endemic countries are associated with NCC lesions in brain identified by neuroimaging [13]. NCC may also act as a risk factor for stroke [14] and migraine-type headaches.

There is an immense burden of epilepsy in the developing countries like India, than developed countries like the West. Three quarters of the estimated 50 million people with epilepsy live in the poor countries of the world and 94% of them remain untreated [6, 69]. But little is known about the causes of epilepsy in developing countries. According to many studies from Latin America, infestation of brain with the larval stage of the parasite, *T.solium* is found to be the important cause of epilepsy in developing countries [65, 62.]. Data regarding the prevalence of epilepsy due to NCC is unavailable in India though in some areas it is reported more frequently [37].

Neurocysticercosis is of great economic relevance, resulting from the cost of medical treatment, lost working days, and losses due to livestock condemnation [11]. It a disease of great economic loss especially

in pig farming communities. The pig production is markedly affected due to cysticercosis , resulting in condemnation of live pigs or carcasses at meat inspection and reduction in their commercial value[60].

Diagnosis of cysticercosis remains a major challenge. NCC is difficult to identify and treat as no diagnostic test identifies all cases of cysticercosis and also each test identifies a different group individual [23]. Diagnosis requires brain imaging, a technology commonly unavailable in resource-poor areas, and clinical diagnosis is unreliable, as the manifestations are diverse and non-specific [11].

The pleomorphic manifestations of the disease and unpredictable response of the host to the cysticerci , make this an intriguing disease. Although theoretically easy to control and declared eradicatable, cysticercosis remains a neglected disease due to the lack of information and lack of awareness of the burden of the disease in the endemic areas. World Health Organisation (WHO) has now declared cysticercosis as the "major neglected disease" [66].

India being a developing country, with large populations below the poverty line, neurocysticercosis is highly prevalent here. The disease is known to have existed in India for several years. A study done by Dixon and Lipscomb in 1961 , in 450 British soldiers who served in India ,

brought to light the latency of the disease and other features. But it did not draw attention to the disease burden in India. There is little epidemiological data on the prevalence of taeniasis , human or porcine cysticercosis in India. Hence, in view of the burden of epilepsy patients in our country and the significant contribution of NCC to this , this present study was undertaken to estimate the seroprevalence of Neurocysticercosis among epilepsy patients in Chennai.

AIMS AND OBJECTIVES

1. To study the prevalence of anti cysticercus antibodies to *T.solium* in the serum of the epilepsy patients

2. To estimate the proportion of NCC cases in the epilepsy patients attending our hospital.

3. To compare the efficacy of the serological and imaging methods in the diagnosis of Neurocysticercosis

4. To compare the efficacy of Enzyme linked immunosorbent assay (ELISA) and Enzyme immune sorbent assay (EITB) for the detection of anti-cysticercal antibodies to *Taenia solium* .

REVIEW OF LITERATURE

Cysticercosis is the most common parasite of the Central Nervous System (CNS). It is caused by the larval stage of the tapeworm ,*Taenia solium* .The word *cysticercus* is derived from Greek word , '*kystis'(*cyst) and '*kerkos'(tail)* because of its appearance.[77]

HISTORY [72]:

Adult tapeworms were first recognized in human feces by the Egyptians which was probably, *T.saginata*. It was called by various names like 'flatworms' as Hippocrates, Aristotle, Theophrastus called , meaning band or ribbon worm. Whereas Romans named it 'lumbricatus latus' meaning broad or wide worm. The parasitc nature of the disease was demonstrated by Malphigi in 1697. The life cycle of *T.solium* was defined by Van Beneden who fed a pig with eggs from human *T.solium* and found numerous cyst in the muscles. Aristotle and Aristophanes were the first to describe cysticercosis in pigs in 3rd century BC . Parunoli noticed it in humans in 1550. There has been references to this disease in our ancient medical literature of '*Charak Sanhita'*. In India the disease was was first identified in Madras after the death of a patient due to seizure. On autopsy the patient was found to be infected with cystic stage

of the parasite. High rate of new onset epilepsy related to cysticercosis in the British army deployed in India has been described by Mac Arthur,1934 [32].

MORPHOLOGY & STRUCTURE :

Larval cestodes belong to the class Platyhelminthes, which are acoelomate metazoan with an elongated dorsoventrally flattened body in adult stage and vesicular bladder in larval stage[72]

Adult Worm :

It is a white, ribbon like, flattened , segmented worm measuring 2-3metres in length[71]. Body of adult worm is divided to head (scolex), neck and chain of segments (strobila). The head measures the size of pinhead and has four pairs of suckers and a rostellum with double crown of hooks.

The characteristic feature of the morphology of the adult worm is that it lacks mouth or digestive cavity . The strobila is made of repeated units or proglottids , proximal segments being immature (lack developed sexual organs) and distal segments being mature (with fully developed sexual organs). The terminal segments are gravid and filled with nearly 30,000-50,000 eggs and the uterus has 5-10 lateral branches. These gravid

segments break off from the strobila and are passed in the faeces as chain of 5-6 segments.[72]

Eggs :

The eggs are released from the gravid segments. They are spherical measuring 31-43µm in size and bile stained [10].The egg is surrounded by thin, outer transparent shell, which represents remnant of the yolk mass[3] . The oncosphere (embryo) is surrounded by the embryophere which is formed by contiguous radial blocks, which gives the radial appearance to the egg. The onchosphere contains six hooklets (hexacanth embryo) , measures 14-20µm and is infective to cattle and humans[71].

These eggs are similar in size and shape to those of *T.saginata, Echinococcus* and *Multiceps.* Hence these eggs cannot be differentiated by light microscopy [71]

Cysticercus cellulosae **(Taenia cyst)***:*

It the larval form of *T.solium* and is infective form of the parasite to man. The cyst is a small , oval and fluid - filled milky white bladder-like structure. It measures 3mm-15mm and appears translucent , the scolex seen as small , eccentric single dense white body [71]. The cysticercus has

two chambers; the inner chamber containing the scolex with the spiral canal and the outer chamber containing the vesicular fluid, nearly 0.5ml .

Two morphological types of cysticerci are seen ; cellulose and racemose. The cellulose cysticerci are small, spherical or oval ,with vesicles measuring 0.5-1.5cm. The racemose cysticerci are large, round or lobulated with delicate walls. It measures upto 10cm – 20cm and may contain 60ml of fluid.

Life cycle [71]:

Definitive host : man

Intermediate host : pig , occasionally man

Man acquires infection by ingestion of undercooked pork infected with *cysticerci.* In the intestine the muscles are digested and the *cysticerci* released. The protoscolex evaginates and attaches to the intestinal wall with the help of suckers and hooklets. The adult worm develops from it, forming new segments at the caudal end . The mature adult worm, which develops in 62-72 days , contains the fertilised egg. These eggs are released into the intestine intermittently , which is extruded in the faeces.

The pigs get infected by ingestion of the eggs or the gravid proglottid segments in the human faeces. The larvae hatch out of the eggs

and attach to the intestinal mucosa. It invades the mucosa and vessel walls in 24-72hours, carried by the circulation to distant sites like muscle, brain etc. It develops into c*ysticercus* form at these sites in 9weeks - 10 weeks , remaining viable for upto 8 weeks.

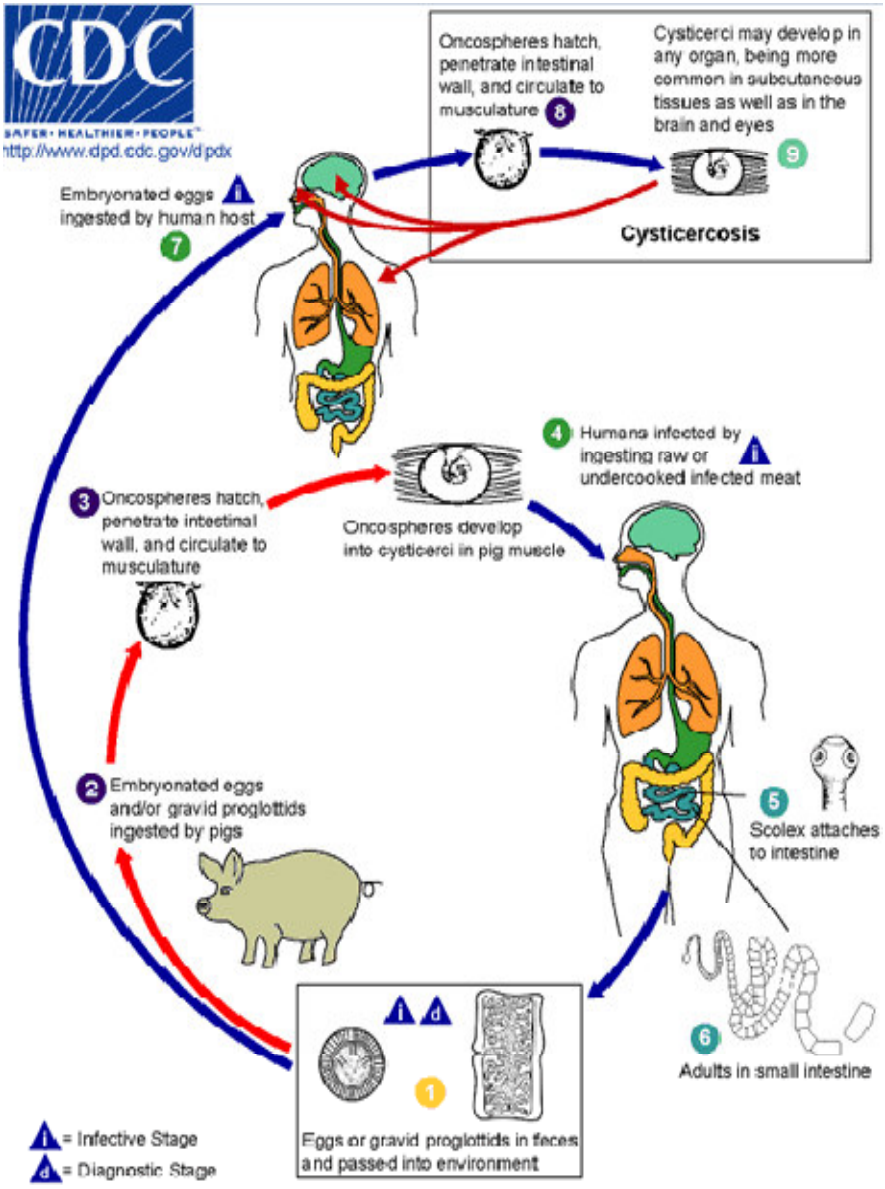


Figure:1 **LIFE CYCLE OF TAENIA SOLIUM**

Occasionally humans become intermediate host by ingesting eggs present in the uncooked vegetables and contaminated water. The eggs develops in humans , just like in pigs and forms cyst. In humans, cysts are produced in the central nervous system, skeletal muscles, eye and subcutaneous tissues. Cellulose type is the most common in type in the human brain[72].

Transmission:

Humans become infested by the adult worm on ingestion of raw or undercooked measly infected pork .Man becomes infested with the metacestode on ingestion of the eggs present in the contaminated drinking water, raw vegetables or from the stool of tapeworm careers [68]. Human to human transmission occurs under poor hygienic conditions when eggs from carriers contaminate the environment and food[36].

Pathogenesis & Pathology :

The adult worms are less pathogenic and remain asymptomatic. They cause minimal irritation and inflammation of the intestinal mucosa [71].

The cysticerci are more pathogenic and produce more serious disease called cysticercosis.

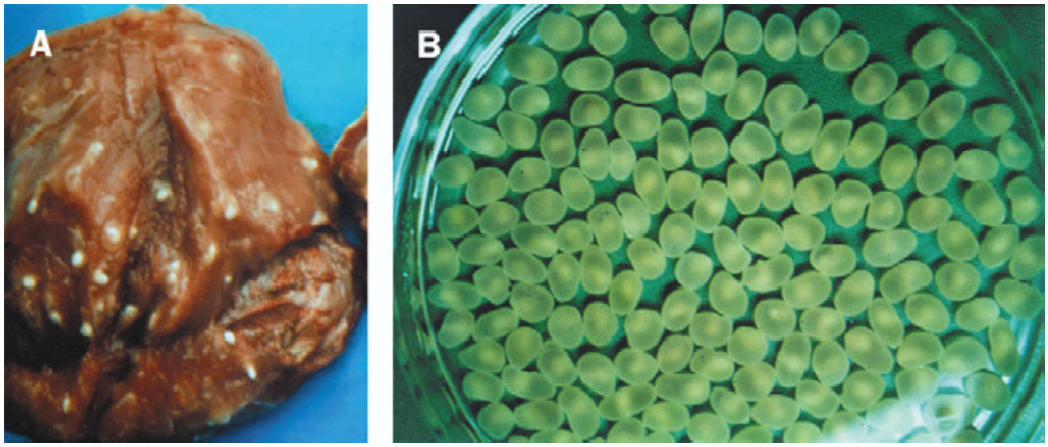


Figure 2: Cysticerci
(A): as seen in infected pork. (B): excised into a Petri dish. The white dot in each cyst corresponds to the scolex. (Courtesy:The LANCET, Vol 361)

Different stages of the Taenia cyst :

Morphologically , four stages of development and regression of the cysticercus in the CNS are recognized.

i. Cystic or vesicular stage – viable cyst composed of well defined , fluid-filled membrane , containing scolex

ii. Degenerating , colloid or Granular stage – corresponds to parasite necrosis and associated inflammatory process.

iii. Nodular stage characterised by fibrosis . This stage can be macroscopically identified as a nodule , smaller than the bladder in the preceding stage.

iv. Calcified granuloma formed by the subsequent calcification of the nodule. The inactive calcified nodule develops within 5 years of infection.

In the brain, the cyst evolves through several stages of development. In the first stage there is intense inflammation surrounding the cyst as it migrates. The disease remains mild or inapparant in this stage .In the second stage the cyst secretes fluid and becomes fluid filled. The larvae develops within it over several weeks. The developing larva secrets a serine protease inhibitor and this favours the cyst from the evading the host immune response. In the third stage , the cyst becomes surrounded by intense host inflammatory reaction. The larva degenerates and the cyst gets filled with caseous material. This stage is associated with a release of cyst contents and antigens into the cerebrospinal fluid (CSF) and serum. In the final stage, the cyst is replaced by fibrotic tissue which is followed by mineralization. This results in an inactive calcified nodule and develops in 5 years of infection.[34]

Immune response[72] :

Anti cysticercus antibodies of several types have been detected but the most frequently found isotype being IgG , subtype IgG2b, IgG2a and

IgG1[28]. It has been detected in the CSF, serum and saliva . But IgG has not been detected in all compartments of the same patient. In the CSF IgM response has been frequently detected than IgG or IgA antibodies. These antibodies are not helpful in controlling the disease, but protects the host from further re infection by the cysticerci[71]. The presence of IgG antibodies indicates the chronic and long term nature of the disease . The antibodies produced are heterogeneous : As seen in Western blot and immune electrophoresis in which upto 30 and 8 antigens have been recognised respectively. The humoral response is generally greater in patients with multiple cyst than with single cyst infection. The intensity and duration of the disease directly correlates with the antibody production [28].

Clinical manifestations :

Two distinct forms of infection caused by the adult worm and cyst are intestinal taeniasis and cysticercosis respectively.

I.Taeniasis :

The adult tapeworm develops in humans after the consumption of infected pork . Taeniasis is characterised by mild symptoms or none at all. Abdominal pain, nausea and loss of body weight have been attributed to the tapeworm infestation.[71,28] Identification of these cases are

important due to the risk of cysticercosis in the carriers and the immediate environment[28].

II.Cysticercosis:

It is caused by infection with the larval stage of the parasite. Humans acquire the infection by feco-oral transmission of the eggs from tapeworm carriers. Hence vegetarians and people who don't consume pork can also acquire cysticercosis[28] .The clinical presentation of the disease depends on the location of the cysticerci, the ocular and neural cysticerci having greater morbidity.

a.Ocular cysticercosis :

Though less common than neurocysticercosis , ophthalmic cysticercosis accounts for 3% of cases. It the most common intra orbital parasitic infestation [28]. The cyst are found freely floating in the vitreous, aqueous humour and sub retinal spaces[71,28]. The patient may present as iritis, palpebral conjunctivitis or uveitis. Visual loss due the retinal detachment from sub retinal cyst can also occur[71,28] . Massive infections with the cyst may even present as proptosis [28] . Fundoscopic examination and orbital ultrasonography are non invasive methods which identifies the parasite [71,28].

b.Neurocysticercosis (NCC) :

Neurocysticercosis (NCC) is the most severe form of the disease and accounts for 60-90% of the cases[71]. The clinical manifestation is highly varied and depends on[71,28]

1. The site of location, viability of the cyst
2. Type of cysticerci[32]
3. Stage of development and involution of the parasite (sudarachnoid, intracerebral, intraventricular, intramedullary)[32]
4. Number of the cyst
5. Host immune response

Two forms of neurocysticercosis are seen based on the location of the parasite in the brain: parenchymal and extra parenchymal cyst.

- **Parenchymal disease** occurs due to the infestation of the brain parenchyma with the cyst. This the most common form seen[32]
- **Extraparenchymal disease** is caused by the cyst located within the ventricles , cisterns , sub arachnoid space or in the spinal cord[71].

The most common site of location of the cyst is parenchymal followed by meninges, ventricles , eye and spinal cord[32].

The presence of cyst is not always associated with symptoms. Although autopsy rates for neurocysticercosis in areas of high endemicity approaches to 2%, the majority of these cases have no symptoms attributable to the infection. It is only when the cyst degenerates and the host inflammatory response occurs, the patient manifests with symptoms like seizures. Therefore the symptoms in NCC may be delayed for several years or the patient remains asymptomatic [32]. Symptomatic NCC may present as follows:

1.Convulsions and seizures :

Epileptic seizures are the common form of presentation[71,28,32,77].Seizures occur in 50-80% of the patients with parenchymal brain cyst or calcification but is uncommon in other forms of the disease. In endemic areas , recent onset on seizures in a healthy young adult ,teenage or middle aged individuals strongly suggests NCC . Seizures, due to NCC, are commonly generalized tonic, clonic or simple partial , few cases present with complex partial or myoclonic seizures[49,45,45]. About 50% of these patients presenting with seizure , further develop seizure on follow up (epilepsy) [28]. Some cysts escape

the host immune response and remain asymptomatic (asymptomatic NCC) . This form is seen in the endemic areas and is caused by the cyst in the second stage of development[34,32].

2.Intracranial hypertension :

NCC can present with signs of raised intracranial pressure , hydrocephalus or both in 20-30% of the cases[28] . It is caused by the cyst located within the cerebral ventricles or basal cisterns causing obstruction to the flow of CSF by various mechanisms – obstruction caused by the parasite itself , ependymal inflammation or residual fibrosis. Headache , vertigo , and altered mental status are the presenting symptoms[71].

3.Meningitis :

Cysts of intra medullary spinal cord can produce motor or sensory disorders.

4.Psychiatric manifestations :

Some patients may present with psychiatric manifestations detected by poor performance on neurological testing and severe dementia[51]. Psychotic episodes characterized by confusion, paranoid ideation, psychomotor agitation, and violent behaviour are other reported manifestations [30].

c. **Subcutaneous cysticercosis :**

It presents as small, painless , subcutaneous nodules most commonly located in the arms and chest. After few years it becomes inflamed and painful followed by gradual resolution. Subcutaneous cysticercosis is commonly seen in Asia and Africa.

Factors for transmission :

Increased consumption of pork and human migration has increased the spread of the disease from the endemic rural areas into urban areas. Livestock raising practices that allow free roaming of pigs is the greatest risk for pigs for acquiring cysticercosis[56]. Lack of environmental and personal hygiene, improper meat inspection , clandestine slaughtering of pigs are other factors favouring the transmission of the disease[55].

EPIDEMIOLOGY

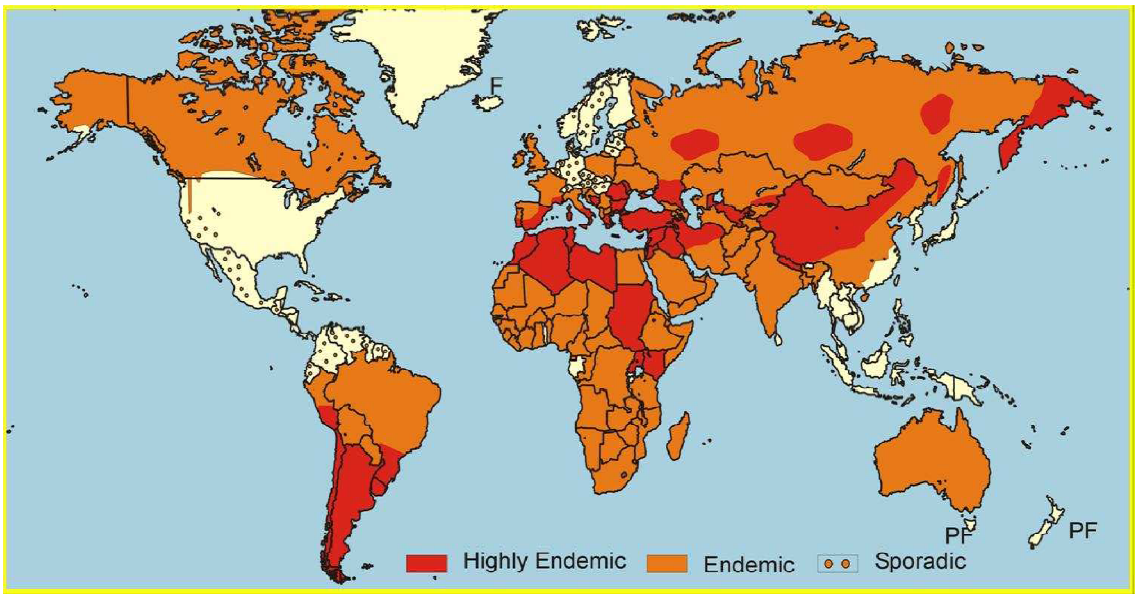


FIGURE 3 : Map of areas affected by neurocysticercosis and cysticercosis . From the World Health Organisation (WHO) , (Geneva, Switzerland)

Nearly 50 million people are known to be infected by Taenia solium worldwide and it is found more common in pork eating communities . The disease is more in developing countries with poor sanitation and hygiene and in developed countries with a higher rate of immigration from endemic areas[28] .

Global :

Taenia solium infection is endemic in Latin America,India,China , Eastern Europe and Sub Saharan Africa[71,51] . Areas with highest prevalence include Latin America and Africa. In some regions in Mexico prevalence as high as 3.6% of the general population has been reported . Seroprevalence ranging from 4.9%-24%[71] has been seen in Latin America. A WHO report says that the average prevalence of this disease in China is about 0.11%[18] . It has been estimated that nearly 1.26 million people live with taeniasis and about 3-6 million people with cysticercosis[18]. Epilepsy prevalence in SSA ranges from 5.2 to 74.4 per 1,000 persons. Even developed countries like Canada and US report of cases of NCC , mainly due to the immigrant population[66] . In some regions like Guatemelia, Peru high seroprevalence of 39% have been reported in humans[46,18].

India:

Cysticercosis has been designated as a "biological marker"of the social and economic development of a community. The disease is prevalent in all states of India, although the prevalence varies between the states. In India all biological and environmental factors , favourable for the transmission of *T.solium* exist. Studies in endemic regions of India reveal high burdens of infection and symptomatic NCC[66].

In a community study in southern India, active epilepsy was present in 3.83/1,000 persons, and 28.4% of these individuals had NCC detected by CT imaging, the seroprevalence of *T. solium* infection was 15.9%. Extrapolating these figures to the overall population in India suggests that approximately 1 million cases of epilepsy are due to NCC [59]. There are few reports of cysticercosis from Kashmir, which is largely populated by Muslims, and from Kerala, as education and hygienic standards are good in these state. In a study done at Bangalore, NIMHANS, nearly 2% of the epileptic patients studied reported diagnosis of NCC. Between 26 and 50% of all Indian patients presenting with partial seizures are diagnosed with a SCG on the CT scan [40] . Another unusual feature is the low proportion of pork eaters amongst Indian patients , less than 1-2% of patients with NCC admits eating pork and more than 95% of Indian patients with NCC are vegetarians. Serological assays using the enzyme linked immunotransfer blot (EITB) has revealed exposure to the disease in 21.5% of 107 neurological patients attending a hospital in Mumbai[16]. Various studies have showed that the single cyst disease is the most common in India. In a study of 156 pathologically proven cases of cysticercosis from Patiala, Punjab, 88% patients presented with solitary lesion. In a seroprevalence study in Chandigarh, anti-cysticercus antibodies were found in 17.3%. In a community-based

survey of population of 15,000 in a slum area in Ludhiana, Punjab, 114 cases of active epilepsy were diagnosed and EITB assay was positive in 27 of 106 cases with active epilepsy. In a community based study in Vellore district of South India the prevalence of NCC causing active epilepsy was found to be 1.3 per 1000 population. The results revealed high levels of exposure of the population to the parasite and a relatively high prevalence of active infections (4.5% antigen positives) but a low prevalence of NCC causing active epilepsy (0.13%). Cysticercosis seroprevalence among the healthy blood donors from Pondicherry was 6.5% using both antigen and antibody detection methods.

Diagnosis:

The diagnosis of NCC is made difficult due to its polymorphic clinical presentation. Criteria based on radiological, serological and epidemiological factors have been laid for the definitive and probable diagnosis of NCC (Revised Del Brutto criteria et al)

Revised diagnostic criteria for Neurocysticercosis :

Categories of criteria:

Absolute criteria

1. Histological demonstration of the parasite from brain or spinal cord lesion
2. Cystic lesions showing scolex in brain by CT or MRI
3. Direct visualization of the scolex in fundoscopic examination of the eye

Major criteria

1. Lesions highly suggestive of neurocysticercosis in neuroimaging studies
2. Positive serum EITB for the detection anti cysticercal antibodies
3. Resolution of the intracranial cystic lesions after treatment with praziquantal or albendazole therapy
4. Spontaneous resolution of the small , single enhancing lesions

Minor criteria

1. Lesions compatible with neurocysticercosis on neuroimaging studies
2. Clinical manifestation suggestive of NCC
3. Positive CSF ELISA for the detection of anti-cysticercal antibodies

or cysticercal antigens

4. Cysticercosis outside the CNS

Epidemiological criteria

1. Evidence of a household contact with *T.solium* infection
2. Individuals coming from or living in areas where cysticercosis is endemic
3. History of frequent travel to disease endemic areas

Definitive : 1) one absolute; or two major + one minor + one epidemiologic. Probable: one major+ two minor; one major + one minor + one epidemiologic; three minor + one epidemiologic

A finding of eosinophils in CSF indicates neurocysticercosis. But other routine diagnostic test are of little help in the diagnosis .Thus serology and neuro imaging form the main investigative techniques in diagnosing neurocysticercosis.[28]

Serological techniques :

A wide range of serological tests have been used in diagnostic and epidemiological studies of cysticercosis . But, most of these studies use the unfractionated antigens and so report high rates of false positive and false negative results[32] . Serology has mainly screening and confirmatory role and should be used in conjunction with neuroimaging for diagnosis[28]. A study done by Mahajan et al found that IHA test to be more sensitive and specific than CFT. On comparison with ELISA , IHT was found to be more specific for the detection of antibodies in CSF while cross reactions were observed with ELISA.[40] In another study from Pondicherry, South India, the co-agglutination test (Co-A) was moderately sensitive and specific for the diagnosis of cysticercosis.[16] These tests are used for the diagnosis of NCC and for the epidemiological studies of NCC[71].

ELISA:

IgG is the predominant antibody detected in patients with cysticercosis.; IgA,IgE and IgM antibodies are of little value in the diagnosis and cannot be correlated with the patient's clinical condition[20]. ELISA can be used for the detection of antibodies in serum and CSF but detection of antibodies in CSF provides better reliability. The test has low sensitivity

of 75% and moderate specificity of 85%. The major disadvantage in serodiagnosis using ELISA is cross reactions occurring in patients with coenurus , *Echinococcus* , *T.saginata*, filariasis[72,28,20]. For this reason it is much more useful when applied to CSF than serum , with the drawback of pain and invasiveness associated with it. An ELISA for the detection antigens secreted by viable T.solium metacestodes used on CSF samples from Peruvian patients have demonstrated positive correlation between antigen levels and the number of live cysts detected by CT and EITB results[20]. Several methods based on ELISA have been established like DOT ELISA, Avidin- Biotin ELISA, dry blood paper ELISA and monoclonal antibody ELISA (MacAb-ELISA). All these newer methods show higher sensitivity and specificity.

Enzyme Immuno Transfer Blot (EITB) :

EITB assay using purified fraction of glycoprotein (Gp) is highly specific (100%) and sensitive (98%) for the detection of antibodies both in CSF and serum[72,28,19]. The presence of 1-7 glycoprotein bands is considered to be diagnostic for *T.solium* infection[71]. The sensitivity of the test depends on the number of cyst : 98% sensitivity in three or more cysticerci and 65% sensitive for one or two cysticerci[19].Hence the assay is of limited value in children because most of them have single

lesion[72]. Although ELISA is most widely used test , EITB is gold standard in serodiagnosis of NCC [19]. Both serum and CSF can be used as diagnostic specimens but it is more sensitive with serum than CSF[17] . But this assay require reagents , expensive equipments and trained personnel for antigen purification and assay performance than ELISA.

Antibody detection in cysticercosis has two major disadvantages: firstly it indicates only exposure to infection. It does not establish whether the patient is having established and viable recent infection. Secondly the antibody continues to persist in the serum even after the parasite has been eliminated through immune mechanism or following drug therapy[12].

Detection of antigens in serum or CSF indicates viable or active infection. Hence they have a role in therapeutic decision and monitoring .Antigen can be detected in serum or CSF by ELISA using specific monoclonal antibodies . These antigen detection tests are highly sensitive and specific[71].

Neuroimaging :

Because of the demonstration of important features like number, location and the stage of the cyst , imaging forms an important modality in the diagnosis of neurocysticercosis (NCC). Currently,CT and MRI are

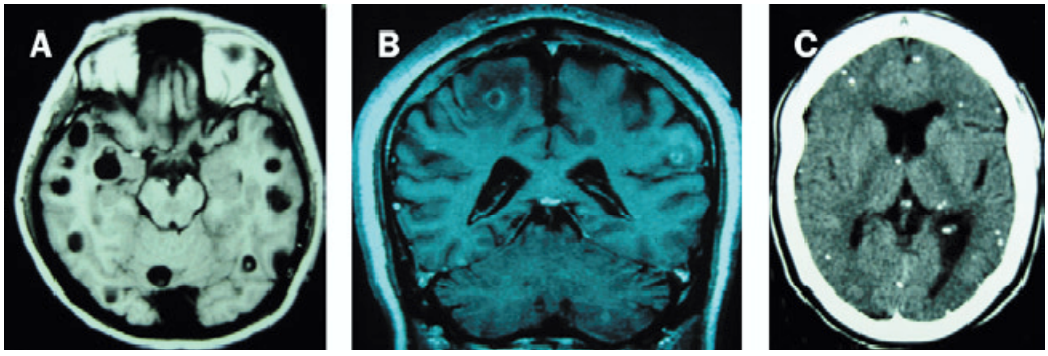


Figure : **Neuroimaging**
MRI of viable (A) and degenerating (B) cysts, and CT of calcified cysticerci (C).
(Courtesy: The LANCET, Vol 361)

the major imaging techniques[28] . On neuroimaging four stages of cyst formation have been described.

1. Vesicular stage – CT showing hypodensity containing a hyperintense small scolex along with nonenhancing or mildly enhancing cyst wall
2. Colloidal vesicular stage – appears as ring enhancing cystic lesion with hypointense cyst wall and surrounding edema
3. Granulomatous stage – cyst wall retracts and forms granulomatous nodule appearing as enhancing nodule with surrounding mild edema
4. Calcified nodule – granulomatous lesion is shrunken and is completely calcified. Appears as single or multiple calcified nodule

Computed tomography (CT) :

It demonstrates the cyst and granuloma stages of the parasite .These cysts are solitary or multiple and usually 5-20mm in diameter. These lesions are usually located in the cortex or grey white matter junction[34]. CT shows high sensitivity and specificity of 95% for the diagnosis of neurocysticercosis. But CT images are rarely pathognomic for NCC[28]. The differential diagnosis of cysticercus granuloma is difficult because of the endemicity of neurocysticercosis and tuberculosis [28] . The disadvantage of CT scan is that it cannot differentiate the various stages of the cyst . Also the cysts located in intraventicular and cisternal regions cannot be detected by CT scan[34,28]. CT is considered best for the demonstration of calcified stage[34] .

Magnetic Resonance Imaging (MRI) :

MRI is the best imaging technique for the diagnosis of NCC due to its high sensitivity and its increased image resolution[34]. The degenerating and innocuous (viable) stages of the cyst can be better demonstrated by the parasite. The viability of the cyst can be established from MRI. The hypointense cystic lesions without the surrounding edema indicates viable cyst. Degenerating cyst is characterised by loss of the cyst fluid signal The number and stage of the cyst can be detected by

MRI in contrast to CT scan[34]. It even detects the cysts located in the ventricle and cistern spaces. But the major drawback is the high cost and scarce availability [5] .

Histopathogical diagnosis[2] :

Specific diagnosis of NCC can be made by the demonstration of *cysticerci* in biopsy obtained from the brain post-mortem. Skeletal cysticercosis can be diagnosed by demonstration of the parasite in the tissue obtained by biopsy or excision of the nodule.

Prognosis and natural evolution:

The natural evolution of the intraparenchymal neurocysticercosis follows favourable outcome with degeneration of the parasite and formation of residual calcified scars .Cysts located in the sub arachnoid space have high morbidity and mortality due to the parasite growth causing increased intracranial pressure , arachnoiditis, hydrocephalus and other complications[28]. Seizure recurrence is high following the first acute symptomatic seizure related to the persistence of active brain lesions. Overall recurrence rate is 40% in one year[19]. Prognosis is best for those patients whom imaging study normalises . Seizure recurrence is reduced in patients with calcifications than those with active lesion[17.61.32].

Treatment :

The therapy of neurocysticercosis varies according to the clinical situation . Various factors that should be taken into consideration during the treatment of the disease include clinical symptoms, location and viability of the cyst, degree of neurological impairment, neuroimaging findings and host's immune response[46] . Asymptomatic NCC with inactive calcified parenchymal NCC or degenerating cysts do not require anti parasitic treatment. Anti convulsant therapy is required in these patient. Active parenchymal cysts are treated with specific anti parasitic drugs, albendazole being the drug of choice[24]. The dose of albendazole is 15mg/kg body weight given in three divided doses for 7 to 28 days. Corticoids is necessary for inflammatory clinical forms such as meningitis and occurrence with vasculitis. Surgical removal may be necessary for giant or racemose cysts and for those producing significant compression syndrome[25].

Control of taeniasis and cysticercosis [2]:

The disease can only be reduced by improving sanitation and controlling domestic pig raising .Consumption of infected pork can be avoided by abattoir inspection and clandestine killing of infected pigs. Human taeniasis cases are important in terms of transmission. Hence,

identifying and treating these population is important in the control of the disease. This can be achieved by the treatment of the tapeworm carriers or the whole population. Health education about the disease and its control by improved sanitation also contributes to the disease control.

MATERIALS & METHODS

Type of study : A descriptive cross sectional study type

Period of study : August 2011 – September 2012

Patient selection :

The study was conducted among the patients attending the neurology clinic of Stanley government medical college & hospital , Chennai for a period of one year from August 2011 to September 2012 . Patients with the complaints of seizure or epilepsy, attending the neurology clinic as in-patients or out patients were enrolled in the study. The study was explained in brief to them in their local language and informed consent obtained . The study group included adult population aged > 12 years , who came with complaint of first episode of seizure (new onset seizure cases) and known epileptic patients who came for follow up (chronic epilepsy). 100 epilepsy patients were selected and serum samples were collected from them to detect the presence of antibodies to *T.solium*.

Inclusion criteria:

Patients with complaints of seizure or epilepsy of both sex irrespective of the treatment .

Exclusion criteria:

1. Cases of epilepsy from metabolic disorder
2. Alcohol withdrawal seizures.
3. Patients with epilepsy secondary to encephalitis or meningitis.
4. Post traumatic epilepsy

Control population :

50 healthy blood donors without complaints of seizure /epilepsy or any such family history were enrolled as controls.

Data collection:

Patient details were obtained from directly from the patients regarding area of residency , occupation . Each participant was interviewed with a questionnaire to analyse the various factors that contribute to *T.solium* infection . Details regarding various potential risk factors like hygienic practices, sanitary conditions , pig rearing practices,

travel to endemic countries, contact with known cases (tapeworm carriers) were obtained . Clinical information regarding the type of epilepsy, frequency , findings of physical examination , radiology and MRI findings were obtained from the attending physicians and medical records. Our centre had MRI facility and most of the patients with complaints of seizure were investigated by this imaging technique by the clinician.

Sample collection:

Stool samples were collected from the study group both from the in-patients and out patients for the detection of *T.solium* eggs . freshly passed stool specimens were collected in wide mouthed, leak proof containers . 3 stool specimen was collected from each patient.

3 ml of blood was collected from each patient from venipuncture of the arm. The samples were centrifuged and serum separated .The serum samples were stored at -20°C until serological tests were done. The serum samples were analysed for the presence of anti – cysticercus antibodies to *T,solium* by ELISA and EITB .

Sample Processing :

Stool examination

Gross examination was done to note the colour , consistency , presence of blood /, adult worm or segments.

Microscopic examination of the feces was done by saline and iodine mount to detect the ova. The stool samples were concentrated by formal ether sedimentation concentration technique . The sediments were examined by iodine and wet mounts to detect the presence of *T. solium* eggs .

1. Saline wet mount :
    - One drop of 0.85% NaCl was placed on a clean glass slide. A small amount of representative fecal specimen was picked up on an applicator stick and emulsified thoroughly
    - A 22X22 mm clean coverslip was placed over the suspension avoiding air bubbles
    - The eggs of Taenia were identified by following features:
        i. Spherical and brown coloured (bile stained) measuring 31-43µm

ii. A thick outer transparent shell and inner embryophore which is brown , thick walled and radially striated

iii. Onchosphere with 3 pairs of hooklets.

2.Iodine wet mount **:**

Iodine wet mount was prepared similar to the saline wet mount , using D'Antoni's iodine .

Processing of Serum specimens :

The serum samples were detected for the presence of antibodies by ELISA and EITB. ELISA was performed with a commercial kit from SCIMEDX , USA. The confirmatory test ,EITB was performed at the department of Microbiology, JIPMER , Puducherry.

ELISA

ELISA for the detection of antibodies to *T.solium* was done with a commercial kit from SCIMEDX corporation, USA. This tests was intended for the screening of serum IgG antibodies to *T.solium* . The test was performed according to the instructions provided in the kit literature.

Principle of the test :

The micro test wells were coated with *T.solium* cyst fluid antigen. During the first incubation period with the diluted patient's sera , any antibodies reactive to the coated antigen bind to the coated wells. After washing to remove rest of the sample , the enzyme conjugate is added. If antibodies have been bound to the well, the enzyme conjugate will then bind to these antibodies. After another series of washes , a chromogen was added. If the enzyme conjugate was present , the peroxidase will catalyse the reaction that consumes the peroxide and turns the chromogen from clear to blue. Addition of stop solution ends the reaction and turns the blue colour to a bright yellow colour. The reaction can be read visually or with an ELISA reader.

Reagents:

1. 96 titre microtitre wells coated with T.solium antigens
2. Enzyme conjugate - protein A conjugated to peroxidise
3. Chromogen - tetramethylbenzidine (TMB)
4. Stop solution - 0.73 M phosphoric acid
5. Positive control - diluted positive rabbit serum
6. Negative control - diluted negative human serum

Preparation of Wash buffer :

Removing the cap the contents of the bottle were added to 475 ml of reagent grade water.

Washing was done with ELISA washer which was set up appropriately.

Preparation of the serum :

Test samples : 5µl of serum was diluted with 315µl of dilution buffer. Dilution of 1:64 was prepared.

Procedure :

1. Wells were broken appropriate to the number of samples to be tested and placed in the strip holder.
2. 100µl (2drops) of negative control was added to the well #1

3. 100µl of positive control was added to the well #2
4. Diluted test samples(patient's serum) was to the rest of the wells 100µl each.
5. The microtitre plate was incubated at room temperature (15-25°C) for 10 minutes
6. The contents were shaken out and washed 3 times with diluted wash buffer
7. 2 drops of enzyme conjugate was added to each well
8. Plate was incubated at room temperature for 5 minutes
9. The plate was shaken to remove the contents and second washing was done 3 times with wash buffer
10. To remove the excess moisture , the plated were slapped against paper towels
11. 2 drops of chromogen was added to each well
12. Plates incubated at room temperature for 5 minutes drops of stop solution was added and mixed by tapping the strip holder.

Reading of results :

Results were read using ELISA reader (Bio rad ,model 680) , set for bichromatic readings at 420/650 nm

Quality control ;

The validation of the kit was done with the controls provided. The values of the control were within the limits provided in the literature . Hence the kit was validated and readings interpreted.

Negative – 0.0 to 0.3 OD units

Positive - 0.5 OD units and above

Interpretation of the results – ELISA Reader

Positive - Absorbance reading equal to or greater than 0.3 OD units

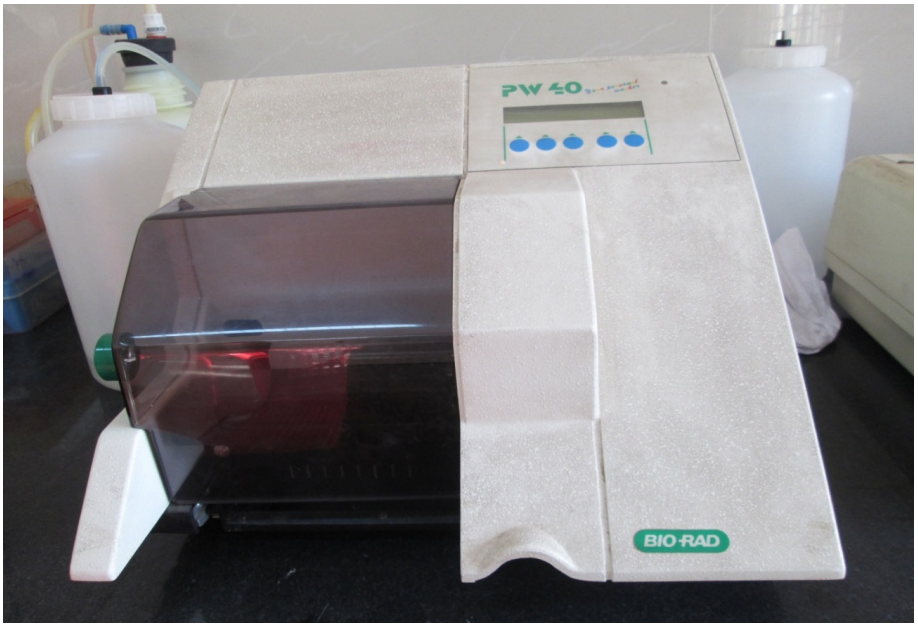
Negative - Absorbance value less than 0.3 OD units

A positive OD reading indicates that the patient may be infected by *T.solium* or a closely related organism (eg.echinococcus)

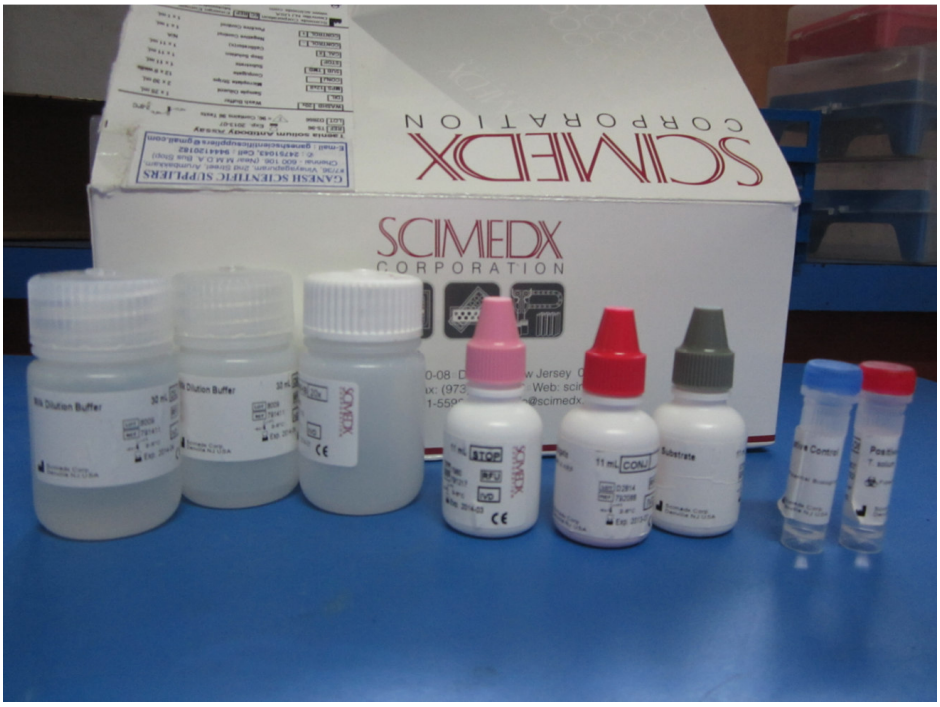
A negative OD reading indicates that the patient has no detectable level of antibodies. This may be due the lack of infection or poor immune response of the patient.

ELISA READER

ELISA WASHER

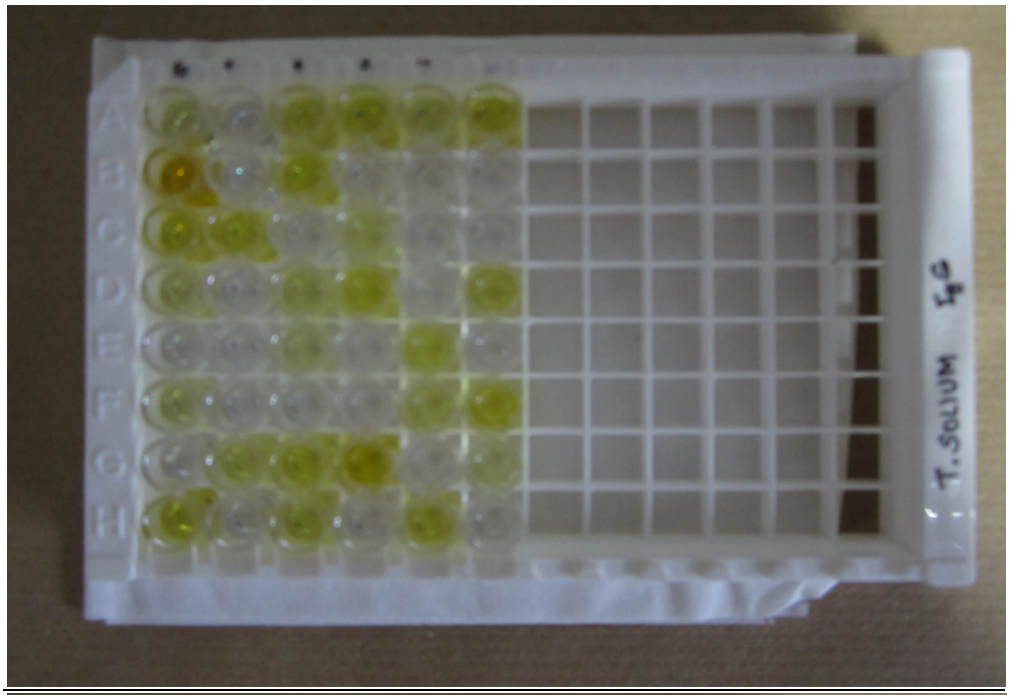


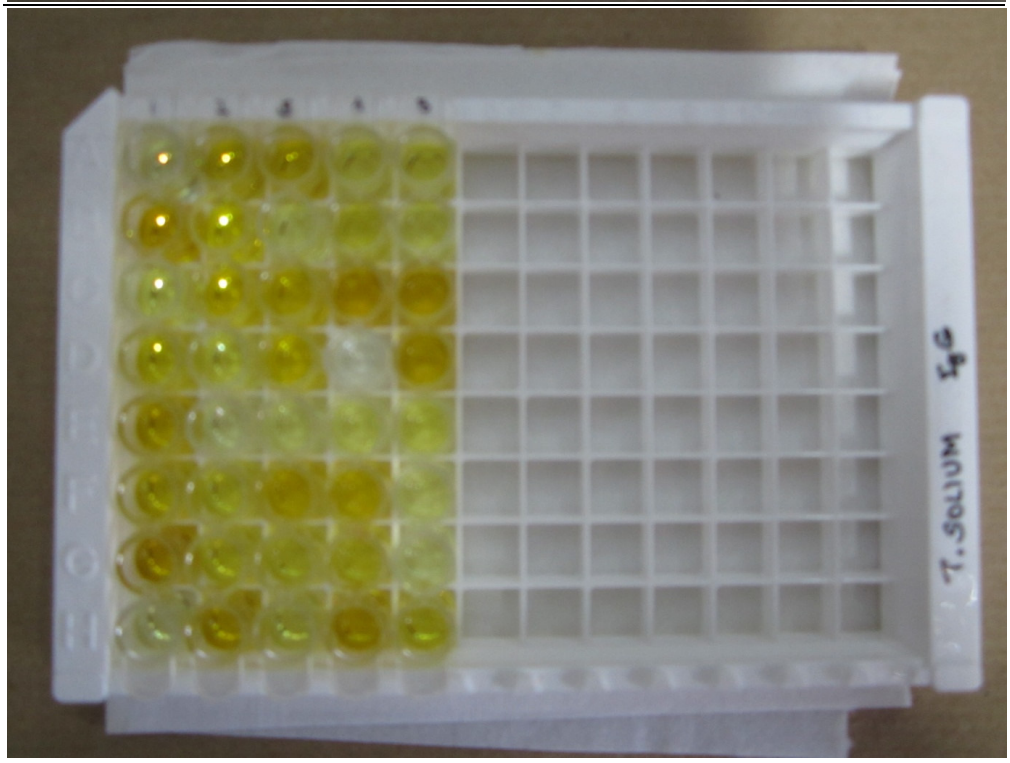
IgG Anti *Taenia solium* Antibody ELISA kit (SCIMEDX Corp)



SHAKING INCUBATOR

ELISA





SODIUM DODECYL SULPHATE-POLYACRYLAMIDE GEL ELECTROPHORESIS(SDS-PAGE)[61,39]

SDS-PAGE is indicated for analyzing the protein profile from the desired source,by characterizing and separating based on the molecular weight of the protein , in this case the antigen.

Principle :

Electrophoresis is the migration of the charged molecules in solution in response to an electric field. In SDS-PAGE the polyacrylamide acts as the support matrix for the running sample.Sodium acrylamide sulphate is an anionic detergent which determines denatures the proteins by wrapping around the polypeptide backbone. The SDS confers negative charge to the polypeptide in proportion to the length. In denaturing SDS-PAGE seperations migration is determined by the molecular weight.

Requirements :

Reagents /Solutions :

Stock solution

1. 2M Tris-Hcl (pH8.8) -100ml

2. 1M Tris –Hcl(pH6.8) -100ml
3. 10%(w/s) SDS
4. 50% (v/v) Glycerol-100ml
5. 1%(w/v)Bromophenol blue-10ml
6. TEMED-(N,N,N',N- tetramethylene –ethylenediamine)
7. 2-mercpatoethanol or Dithiothreitol
8. Glycine

Working solutions

1. Acrylamide stock (30%)-100ml
2. Separating gel buffer (4x)-100ml
3. Stacking gel buffer (4x)
4. 10% Ammonium per sulfate (APS)-5ml
5. Electrophoresis / running buffer (1x)-1000ml
6. Sample buffer -10ml
7. Staining solution-1000ml
8. Destaining solution -1000ml

Procedure :

10% separation gel and 5% stacking gel were used.

Sample preparation :

The glycoprotein antigens were prepared by using metacestodes obtained from the cyst fluid in the naturally infected pigs.

The antigen (20µl) was mixed with the sample buffer (5µl) in an eppendorf and heated at 75-100°C for 2-10minutes .

1. Clean glass slides were assembled by placing the spacers –two on either sides & one along the bottom edge & the whole assembly was tightly with clamps .

2. The glass plates assembled were sealed with melted wax on all three sides leaving the top side , so that the assembly is leak proof.

3. The casting gel were prepared with the components of the separating gel mixture without TEMED & deaerated.TEMED was finally added to the mixture and the gel immediately poured between the glass slides upto 2cm below the notch. The gel was allowed to polymerize for 30-60minutes at room temperature.

4. The gel was overlaid with n-butanol which helps keep the gel surface flat. After polymerisation the butanol was removed and rinsed with water.

5. The stacking gel was prepared and cast over the separating gel as mentioned above and the comb inserted.

6. It was allowed to polymerize for 15-20 minutes. Then the comb was removed carefully and the wells rinsed with distilled water.

7. The casted gel plate was removed from the gel casting stand /clamps and the bottom spacer detached. The gel assembly was placed in the electrophoresis chamber with the notched plates facing the inside . The upper and lower tanks were filled with running buffer.

8. The wells loaded with the antigen (10-50µl) diluted in sample buffer along with a molecular weight markers (5-10µl) .

9. The electrophoresis was run at a constant current of 20mA till the dye front reached the separating gel and then increased to 25mA. The run was stopped when the dye front reached the

bottom of the gel.The side spacer were removed from the glass plates and carefully the gel scooped using the spatula .

10. The gel was stained using the Coomassie staining solution for 1-2 hours & then destained using the deataining solution for overnight in a rocking shaker.

Results & interpretation :

The bands separated according to the molecular weight were visualised directly and checked for the desired bands with the standard marker.

The bands are transferred by blotting (Western) and confirmed by coupling with appropriate antisera containing complementary antibodies for the antigen.

ENZYME IMMUNO TRANSFER BLOT (EITB) [12,27,73]

Western blot or immunoblot (EITB) is the confirmatory test for diagnosing cysticercosis. Immunotransfer blot is the method used to detect a protein immobilized on a matrix. It is a method for identification

of a single protein in a complex mixture following separation based on its molecular weight , size and charge.

Principle :

The antigenic components are first separated by polyacrylamide gel electrophoresis . The separated proteins are transferred from the polyacrylamide gel to a porous membrane and probing this with antibody.antigen antibody complex is then detected using labelled anti-immunoglobulin reagent .Antigens recognised by the antibody appears as bonds on the matrix and by comparison of these with the protein stained antigens allows the identification of antigens recognised by antibody.

Procedure :

Immunblottting is divided into two steps: Transfer of the protein from the gel to the matrix and detection of the epitope with specific antibody. The protein transfer is achieved electrotransfer by semi-dry blotting technique. In this technique the gel - matrix is sandwiched between buffer –wetted filter papers through which current is applied for 10-30 minutes.

Requirements :

Apparatus

1.Electroblotting apparatus

2.Power pack

3.Rocker shaker

4.Whattman 3MM paper (0.2µm)

5.Nitrocellulose membrane (0.22µm) (BIOTRACE ™ NT nitrocellulose transfer membrane , PALL Corporation)

6. Membrane holding forceps

7. Containers for membrane processing

1.Reagents and Buffers :

i. Transfer buffer

ii. Phosphate buffered saline

iii. Substrate buffer

iv. Substrate buffer for horse radish peroxidise

v. Antibody / conjugate dilution buffer

vi. Washing buffer (PBS-T)

2.Destaining solution

3. Ponceau ‘s stain

General assembly of the unit for transfer :

1. The transfer buffer was prepared and equilibrated with acrylamide gel in buffer 20-60 minutes
2. Two pieces of pre-cut extra thick blot paper and pre-cut nitrocellulose membrane was saturated in transfer buffer. The transfer membrane was equilibrated for atleast 10 minutes.
3. The safety cover and stainless steel cathode assembly were removed
4. Pre-soaked sheet of extra thick filter paper was placed onto platinum anode. The surface of the filter was rolled out to remove air bubbles
5. The pre-wetted blotting media was placed on top of the filter paper and air bubbles rolled out.
6. The equilibrated gel is carefully placed on the top of the transfer membrane , aligning the gel on the centre of the membrane .
7. The other sheet of pre-soaked filter paper strip was placed on top of the gel; and air bubbles between the gel and the filter paper carefully removed.

8. The cathode is placed back on the stack. The latches were engaged with the guide post without disturbing the filter paper stack. The unit was plugged to the power supply and turned on. The transfer was run for 60-90minutes at 20V.
9. Following the transfer , the antibody reactivity is visualised with a substrate solution containing diaminobenzidine (6mg/ml in PBS containing 1ml of 30% of hydrogen peroxide)

Immune detection :

Blocking membrane

1.10ml Blocking buffer was added (3% BSA in PBS 7.2)

2. The filter paper was rocked gently for 30 minutes to 1 hour, so that the entire paper was in contact with the blocking buffer

Wash membranes :

The blocking solution is poured off and rinsed with PBS –T thice

First antibody:

1.Patients's serum at appropriate dilution in 10ml of PBS-T

2. Rocking was done gently for 1 hour in shaking incubator

Wash membrane

First antibody was poured off and washing was done with wash buffer (3 times)

Second antibody :

The second antibody was added at appropriate dilution in 8ml 0.5% BSA TBS . Gentle rocking was done for 1 hour.

Wash membrane

The second antibody was poured off , rinsed for 30 minutes with PBS –T in 3 changes.

Develop membrane

1. The PBS-T was poured off from the membrane and then transferred into developing reagent
2. It was rocked gently monitoring development
3. The development was stopped by washing membrane with distilled water for 30minutes in 3 changes

Destaining

1. The staining solution was poured off and sufficient destaining solution was added

2. The filter was rocked gently till the bands were clearly visible and the background clear from the stain

Interpretation :

The sample was considered reactive if it showed reactive bands to one or more glycoprotein antigens.

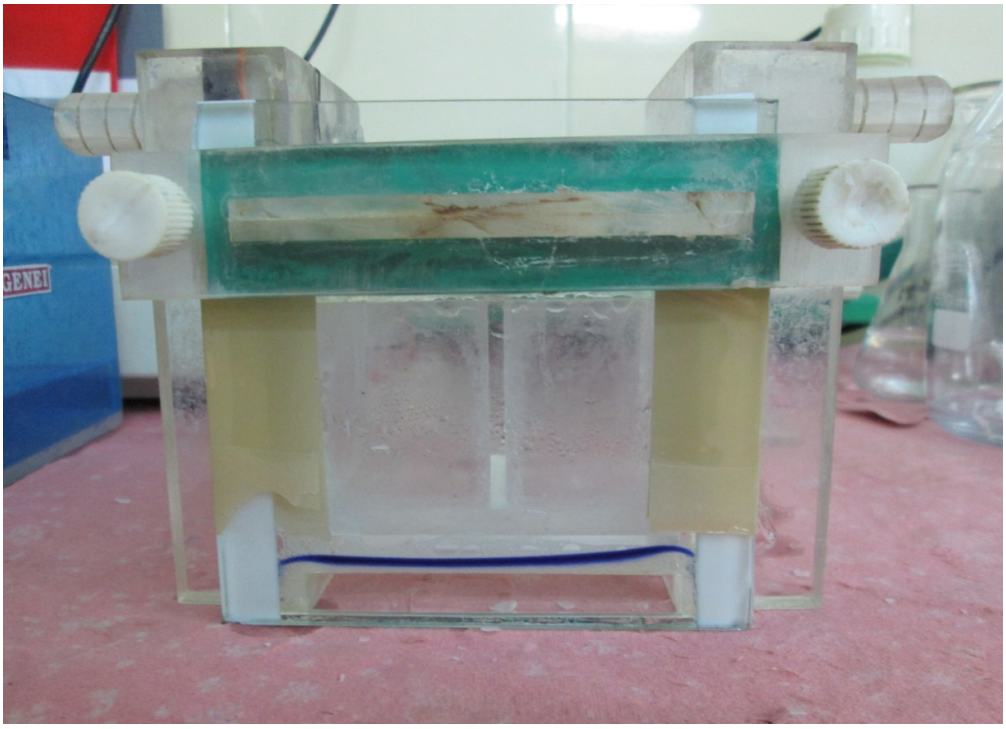
Ethical consideration :

The study protocol was submitted to the ethical committee of our institution. Ethical and research clearance was obtained for the study on 2011. With the permission from the HODs of the respective departments , the study was started . Informed consent was obtained from the patients before enrolling in the study.

Statistical analysis :

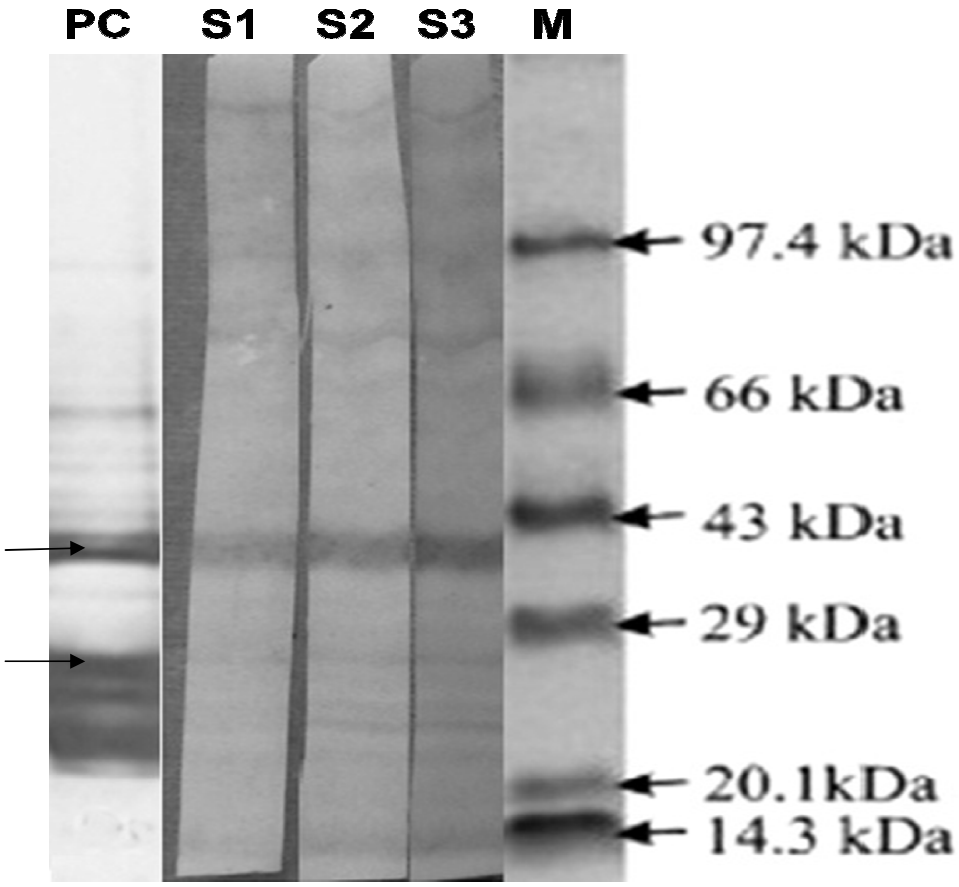
Statistical analysis was done with chi-square test or student t test where necessary.

SDS – PAGE ELECTROPHORESIS



TRANSFER UNIT APPARATUS

ENZYME IMMUNO TRANSFER BLOT (EITB)



PC- POSITVE CONTROL ,M- MOLECULAR MARKER,S1,S2,S3 - TEST SAMPLES

Arrow ; REACTIVE BANDS AT 40KDA AND 24KDA BANDS

RESULTS

About 100 cases , with complaints of epilepsy / seizure attending attending the Neurology department with complaints of seizures / epilepsy and 50 healthy controls without history of epilepsy were enrolled in the study , conducted during the period from August 2011 to September 2012 at the Department of Microbiology , Government Stanley Medical college ,Chennai. . Blood samples were collected from all the patients for testing for the presence of anti-cysticercus antibodies. The serum samples were processed by ELISA and EITB. Of the 100 epileptic patients enrolled , 48 patients submitted their stool specimen for examination .3 consecutive stool specimen was collected from 14 cases and the rest were lost in consecutive follow up and had submitted single stool sample.

The results were analyzed as follows :

Table -1

Age distribution among the study and control groups :

Age (years)	**Study group**		**Control group**	
	No.	%	No.	%
≤ 20	29	29	13	26
21-30	33	33	16	32
31-40	18	18	10	20
41-50	12	12	7	14
51-60	6	6	3	6
>60	2	2	1	2
Total	100	100	50	50
Mean age ±SD	28.42±12.76		29.46±13.55	
t-value	0.46			
df	148			
P value	0.46			

Most of the cases in the study group were in the age group of 21-30 years with the mean age distribution of 28.42±12.76 years. In the control group also most of the patients were in the age group between 21-30 years with the mean age group of 29.46±13.55 years . Hence the study group and the control group are comparable.

Table -2

Gender distribution among the study and control groups :

Age (years)	**Study group (n=100)**		**Control group (n=50)**	
	Male %	Female %	Male (%)	Female (%)
≤20	13	16	8(16%)	5 (10%)
21-30	24	9	10(20%)	6(12%)
31-40	14	4	6(12%)	4(8%)
41-50	6	6	4(8%)	3(6%)
51-60	5	1	1(2%)	2(4%)
>60	2	0	1(2%)	0
Total	64	36	30(60%)	20(40%)
Male to female ratio	1.8:1		1.5:1	
Chi square	9.7		0.73	
df	5		5	
P value	0.24		0.73	

The study group and control group were similar in the gender distribution with the male to female ratio in the study group being 1.8:1.

Chart 1**: Age distribution among the study population**

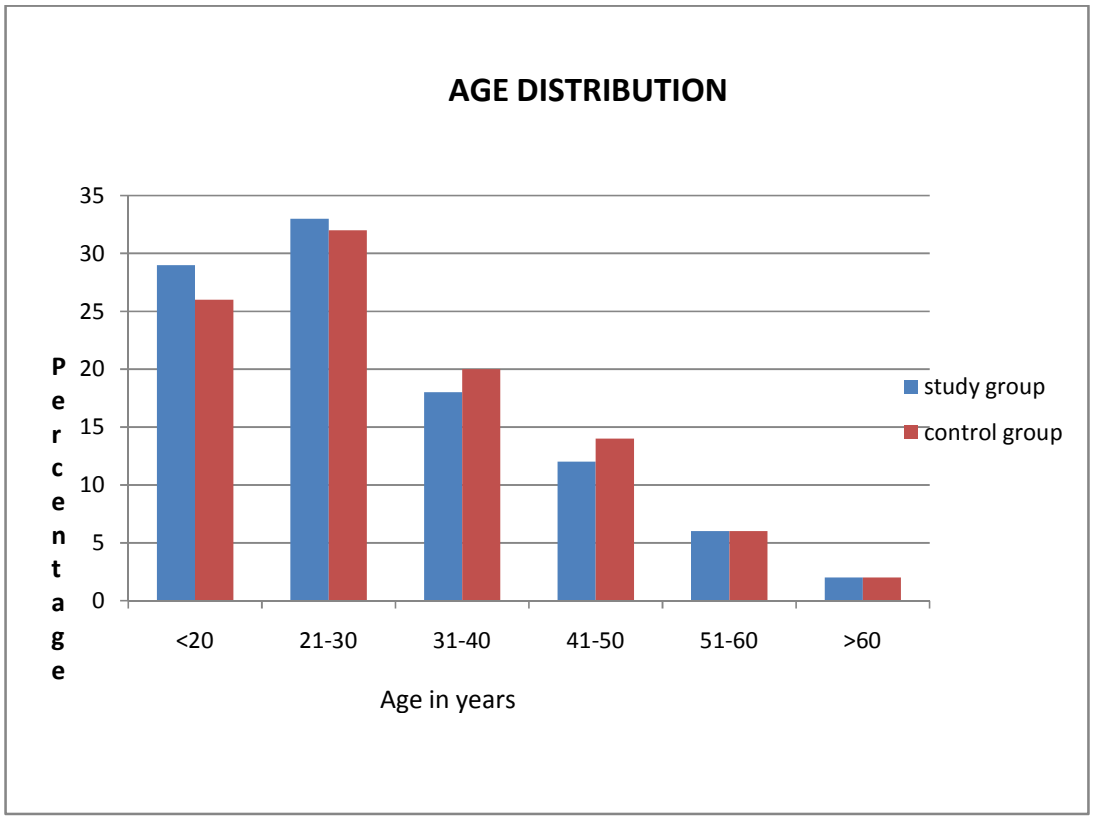


Chart 2: **Gender distribution among the study and control groups**

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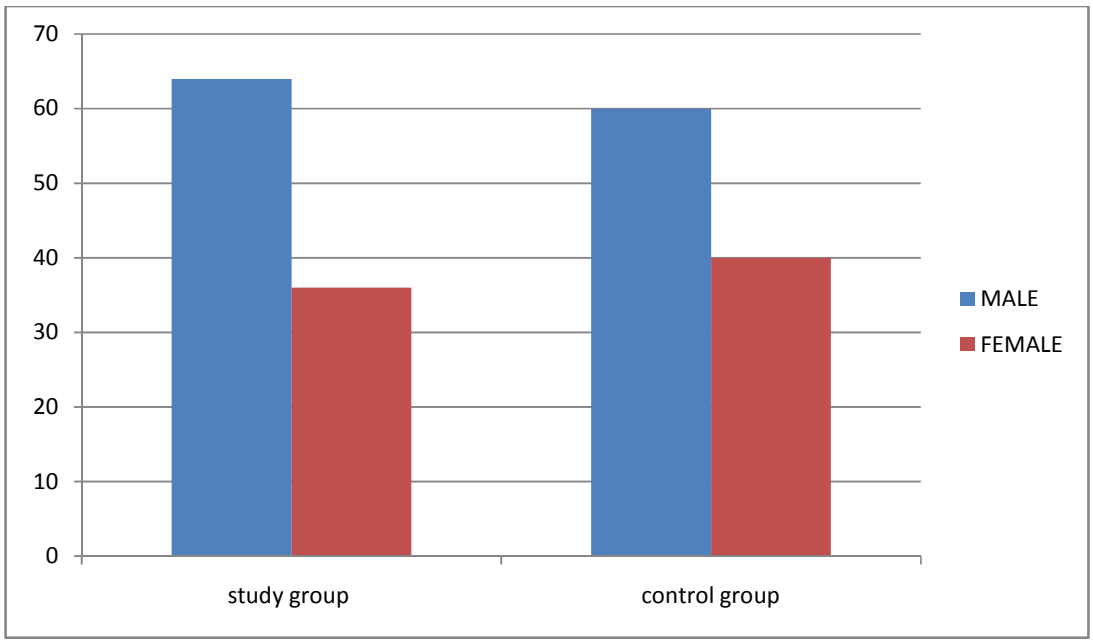


Table – 3

Distribution of epilepsy according to the duration in the study group (n=100) :

Age (years)	**New onset seizure(%) (< 1 year)**	**Chronic epilepsy(%) (>1 year)**
≤20	18	15
21-30	10	21
31-40	6	11
41-50	2	8
>50	5	4
Total (%)	41	59

New onset seizure was more common in ≤20 years age group while chronic epilepsy was common in the age group of 21-30 years.

Chart -3

Distribution of epilepsy according to the duration among the study population

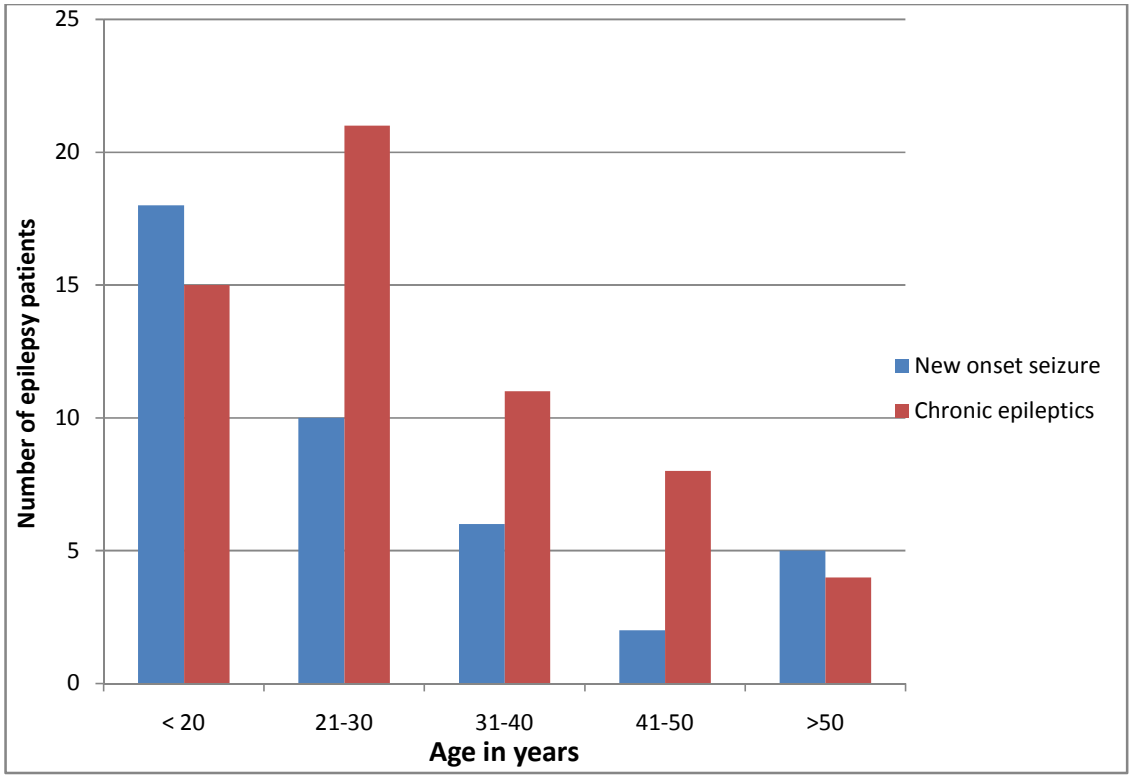


Table – 4

Various types of epilepsy among study group(n=100) :

Type of epilepsy	**Number of patients among the study population**	**Percentage (%)**
GTCS	73	73
PS	27	27

GTCS- Generalised Tonic Clonic Seizures PS- Partial seizures

GTCS was reported in 73% of cases and partial seizures in 27%

Table – 5

Gender distribution of pork consumption in the study group and control group:

Gender	**Vegetarians**	**Non vegetarians**		
		Total	**Pork consuming**	**Non pork consuming**
Study group : n=100				
Male	9	55	10	45
Female	11	25	8	17
Total (%)	20	80	18	62
Control group : n=50				
Mal e	8	22	3	19
Female	4	16	1	15
Total	12(24%)	38(76%)	4(8%)	34(68%)

In terms of the dietary habits, 20% of the study population were vegetarians and 80% non vegetarians of which 18% consumed pork and it was slightly seen more among males than females. 8% of the control group consumed pork of which 3 (6%) were males and1(2%) female

Chart 4: **Various types of epilepsy in the study in the study group:**

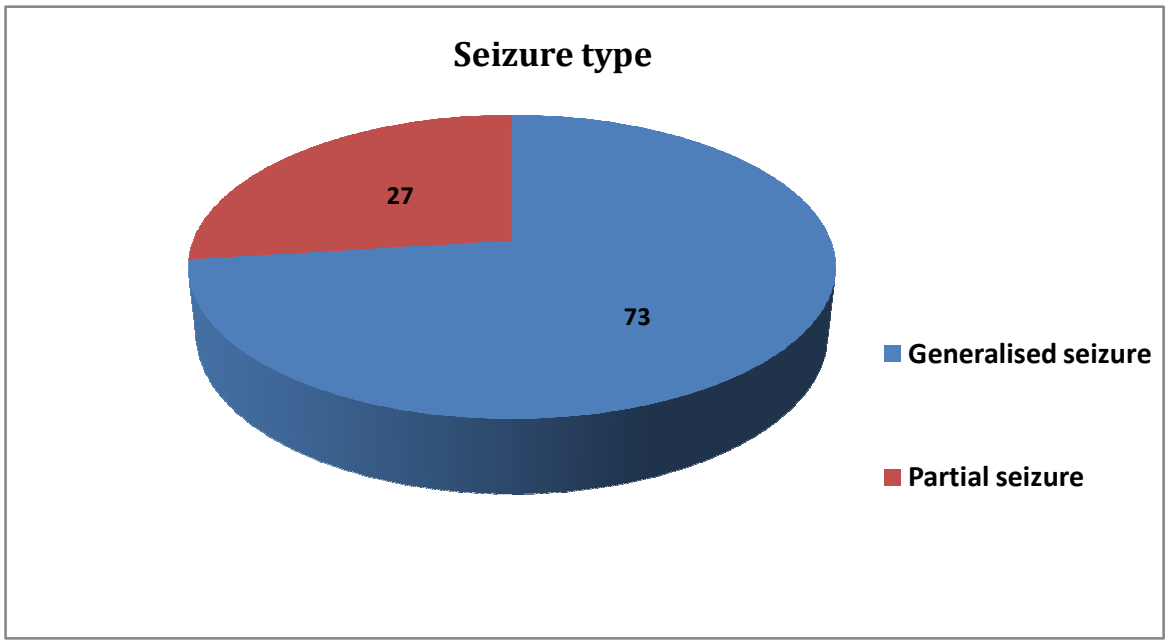


Chart 5: **Consumption of pork in the study group:**

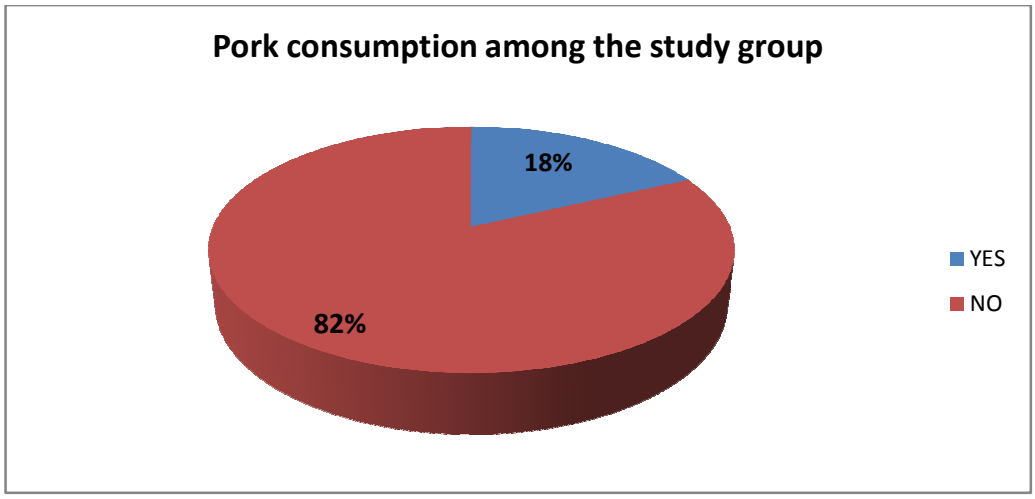


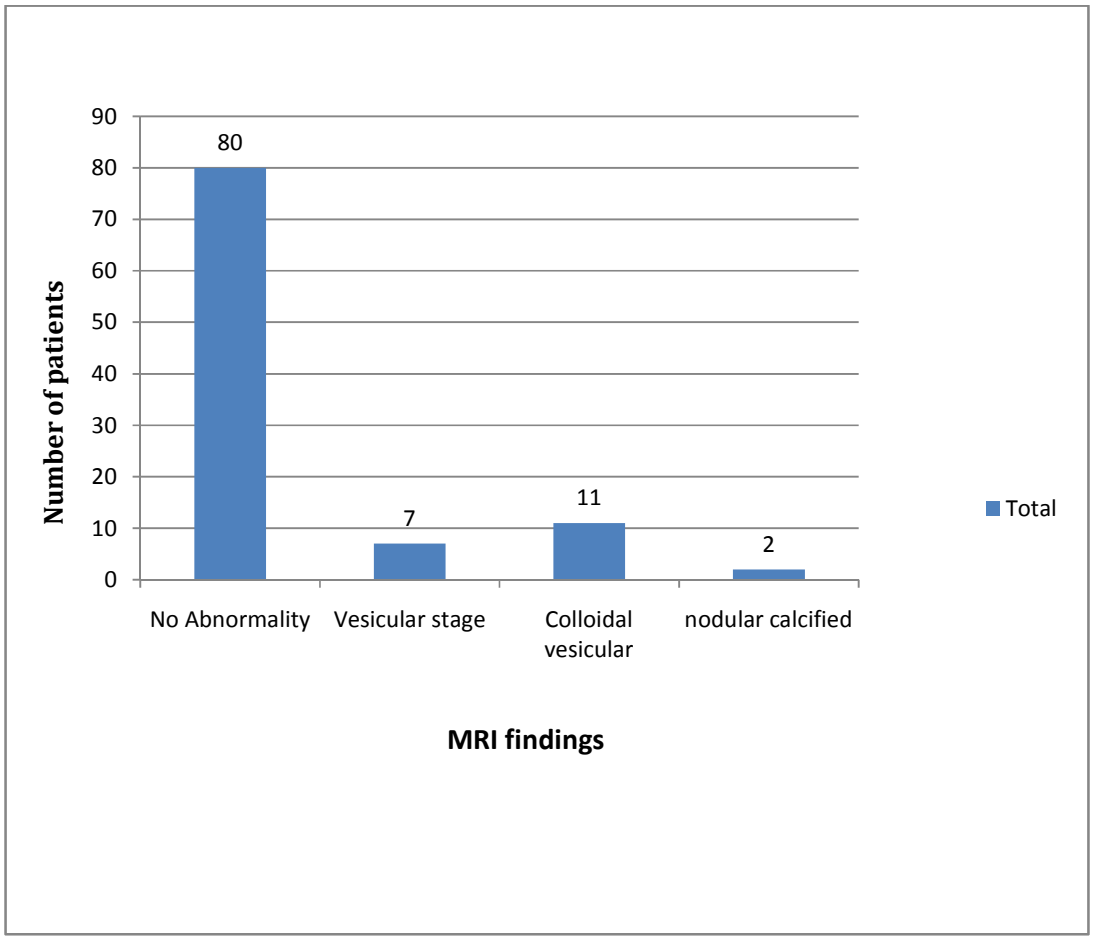
Table – 6

MRI findings in the suspected cases of Neurocysticercosis (n=100)

Age group (years)	No abnormality	MRI findings			
		Total	Vesicular stage	Colloidal vesicular	Nodular calcified
≤ 20	24	6	3	3	0
21-30	31	6	2	4	0
31-40	13	1	0	1	0
41-50	8	4	0	2	2
>50	4	3	2	1	0
Total	80	20	7	11	2
Chi square	13.4				
Df	12				
P value	0.4				

Based on the MRI findings of the study population 20% of the cases were diagnosed /suspected to be NCC . Colloidal vesicular suggestive of NCC was the common finding seen in 11% of the cases , found more in the age group of 21-30yrs. Nodular calcified stage was seen in 2 % in the age group of 41-50 years

MRI FINDINGS IN THE STUDY GROUP :



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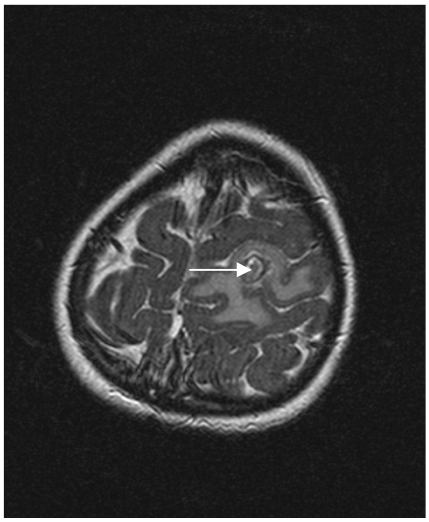
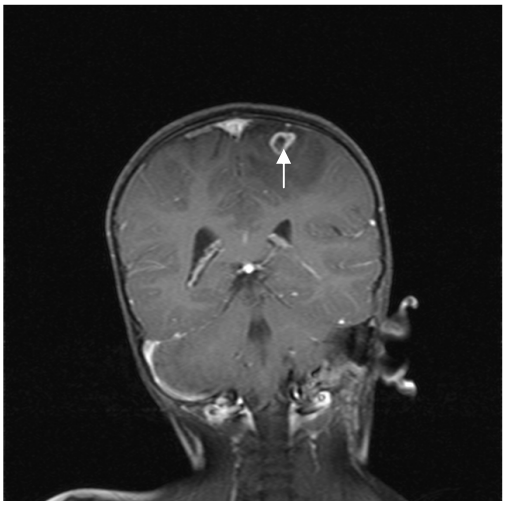
PICTURE 1A



PICTURE 1 B.

PICTURE 1C



PICTURE 1D

PICTURE 1A **AN AXIAL FLUID ATTENUATION INVERSION RECOVERY (FLAIR)**

1B **T2 WEIGHTED IMAGE** , 1C & 1D **GADOLINIUM ENHANCED MRI**

SHOWS A LEFT PARIETAL RIM ENHANCING LESION WITH A HYPERINTENSE MURAL NODULE

Table – 7

Comparison of the two groups from MRI

i.e MRI suspected cases of NCC and normal study

Characteristics	**Category**	**MRI suspected cases of NCC(%) n = 20**	**Normal study** n = 80
Age group	<20	6(30%)	23(28.6%)
	21-30	6(30%)	27(33.8%)
	31-40	1(5%)	17(21.2%)
	41-50	4(20%)	8(10%)
	>50	3(15%)	5(6.3%)
Gender	Male	12(60%)	54(67.5%)
	Female	8(40%)	26(32.5%)
Seizure type	Partial	4(20%)	23(28.8%)
	Generalised	16(80%)	57(71.2%)
Duration of seizure	≤ 1 year	10(50%)	40(50%)
	2-4 years	1(5%)	12(15%)
	5-9 years	1(5%)	10(12.5%)
	≥10 years	8(40%)	18(22.5%)

Majority of the epileptic patients with lesions suggestive of neurocysticercosis in MRI was found in the age group <20 and 21-30 years age groups , predominantly in males . Most of them presented with generalised seizure for less than a year.

Stool microscopy for detection of Taenia eggs**:**

Of the 150 patients , stool sample was obtained from 68 cases i.e 48 from study group and 20 from control group. Wet mount and iodine mount was performed and following results obtained.

Of the 68 stool specimens , *Taenia* egg was detected from none of the cases in study or the control group.

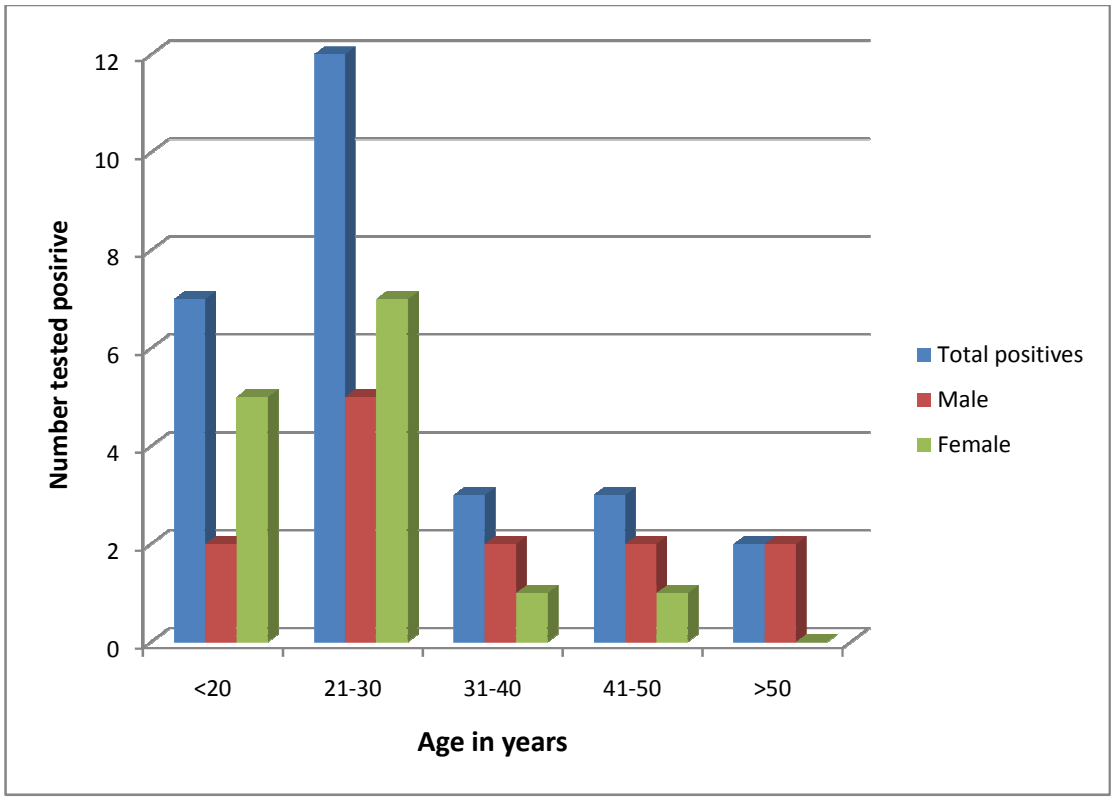
Table -8

ELISA for the detection of anti-cysticercal antibodies to *Taenia solium* :

Age group	No. of samples tested (n=92)		No of samples antibodies detected						No of samples antibodies not detected	
			Total		Male		Female			
	no.	%	no	%	no.	%	no.	%	No .	%
<20	31	33.7	5	5.4	3	3.2	2	2.1	26	28.3
GTCS	21	22.8	2	2.1	0	0	2	2.1	19	20.7
PS	10	10.9	3	3.2	3	3.2	0	0	7	7.6
21-30	33	35.9	10	**10.9**	2	2.1	8	**8.7**	23	25
GTCS	26	28.3	8	**8.7**	3	3.2	5	5.4	18	19.7
PS	7	7.6	2	2.1	0	0	2	2.1	5	5.4
31-40	12	13	4	4.3	3	3.2	1	1	8	8.7
GTCS	9	9.8	2	2.1	2	2.1	0	0	7	7.6
PS	3	3.2	2	2.1	2	2.1	0	0	1	1
41-50	13	14.1	2	2.1	1	1	1	1	11	12
GTCS	12	13	1	1	1	1	0	0	10	10.9
PS	1	1	0	0	0	0	0	0	1	1
>50	3	3.2	2	2.15	2	2.1	0	0	1	1
GTCS	2	2.1	2	2.1	2	2.1	1	1	0	0
PS	1	1	0	0	0	0	0	0	1	1
Total	92		23	**25**	11	**12**	12	**13**	69	75
Chi square	6.10				2.80					
df	4				3					
P value	0.19				0.59					

ELISA detected antibodies in 25% of the serum samples 12% of males and 13% females among the epilepsy patients. Of this highest seroprevalence of antibodies i.e 10.9% was seen in the age group 21-30 years and 8.9% presented with GTCS. There is no significant difference in the seropositivity between males and females($p > 0.05$)

Chart 7: **RESULTS OF EITB**



SDS –PAGE AND EITB:

Analysis of the whole cyst antigen after seperation by SDS –PAGE revealed distinct protein bands of molecular weights ranging from 106kDa to 10kDa. The major antigenic peptides were found to be 76kDa, 68 kDa, 40 kDa, 32kDa,24 kDa and 18 Kda. On immunoblotting with sera from the patients showed reactivity at 40kDa and 24kDa bands.

Table-9

Enzyme Immuno Transfer Blot (EITB) for detection of antibodies to *Taenia solium*:

Age	EITB reactive	%
Epileptic patients n=100	27	27
Healthy controls n=50	3	6

EITB detected antibodies to larval stage of *T.solium* in 27% of the epileptic cases. 6% of the control population were reactive. There is significant association of the epilepsy with the presence of anti-cysticercus antibodies in the serum.

Table - 10

Distribution of Age , gender , seizure type and duration among the seropositives (n=27):

Characteristics	**Category**	**EITB n=27**	**P value**
Age group	<20 21-30 31-40 41-50 >50	7(25.9%) 12(44.4%) 3(11.1%) 3(11.1%) 2(7.4%)	0.99
Gender	Male	12(44.4%)	0.811
	Female	15(55.6%)	
Seizure type	Partial	9(33.3%)	
	Generalised	18(66.7%)	0.36
Duration of seizure	≤ 1 year	12(44.4%)	
	2-4 years	6(22.25)	
	5-9 years	4(14.8%)	0.26
	≥10 years	5(18.5%)	
MRI	Suspected NCC cases	8(29.9%)	0.6

The comprehensive clinical details of the antibody positive cases is given in the above table. The presence of antibodies detected by EITB was high in the age group of 21-30 years . EITB tested slightly more positives in females (55.6%) than males (44.4%) , the male to female ratio being 1: 1.3 and it was not statistically significant ($p>0.05$). Generalised tonic clonic seizures was detected among 66.7% of the cases in which antibody was detected. Antibodies were detected more in the cases presenting with seizures for less than 1 year duration.

Table - 11

Association of potential risk factors and the seropositivity by EITB:

Risk factors	Seropositive	Seronegative	p value
Pork consumption n=18	13(72.2%)	5(27.8%)	0.07
Lack of personal hygiene & toilet facilities n=45	20(44.4%)	25(55.6%)	

Analysis of the distribution of the potential risk factors between the seropositive and seronegative groups, showed that about 72.2% of the pork consuming population had antibodies to *T.solium* . Among those with poor toilet facilities, antibodies were detected in 44.4%.

Table - 12

Comparison of the imaging and serology results :

EITB	MRI suspected cases of NCC (n=20)	MRI without Abnormality(n=80)
Antibodies present	10(50%)	17(21.3%)
Antibodies absent	10(50%)	63(78.6%)

On comparison of the imaging and serology results by EITB ,50% of the cases who had imaging findings suggestive of NCC were reactive in EITB. Of the patients with normal imaging study , antibodies were detected in 21.3% .

Table – 13

Comparison of ELISA positives (n=23) and EITB positives (n=27)

	Both EITB&ELISA positive	EITB only positive	ELISA only positive
No of samples	17	10	6

In 17 cases antibodies were detected by both ELISA and EITB . In 10 cases antibodies were detected by EITB only . False positivity of ELISA was seen in 6 cases.

Table-14

Sensitivity and specificity of ELISA in comparison to EITB

Sensitivity	61.5%
Specificity	90.4%
PPV	66.67%
NPV	89.19%

PPV-Positive Predictive Value

NPV- Negative Predictive value

Taking EITB as the standard, ELISA showed sensitivity of 61.5% and specificity of 90.4%.

Based on the radiological and serological findings , applying the Del Brutto et al criteria for the diagnosing neurocysticercosis, 8 cases of NCC was identified - 4 definite and 4 probable cases. The clinical details of these patients is given in the following table.

Table – 15

Definite and probable cases of NCC based on Del Brutto's criteria (2001)

No.	Age /sex	Onset of seizure	Duration	Type of seizure	Pork consumption	MRI	Antibodies EITB
Definite cases							
1.	42/M	New onset	3 days	GS	yes	Granuloma	Present
2.	21/F	New onset	16 days	GS	no	Granuloma	Present
3.	26/F	New onset	6 mon	GS	yes	Multiple REL	Present
4.	17/m	Chronic	2 years	GS	yes	Granuloma	Present
Probable cases							
1.	15/m	New onset	4 months	GS	no	Granuloma	Present
2.	22/M	Chronic	3 years	GS	no	Granuloma	present
3.	50/m	Chronic	3 years	GS	yes	Granuloma	Present
4.	25/f	Chronic	12 years	GS	no	Granuloma	Present

GS- generalised seizure REL- ring enhancing lesion

The definite and probable cases of NCC were in the age between 15-45 years, males slightly more than females. In the definite cases of NCC , most presented with new onset , generalised seizures. Pork consumption was found in 3 of the 4 definite cases. Solitary granuloma was the most common MRI finding in these cases.

Discussion :

Epilepsy due to neurocysticercosis (NCC) is a major public health problem especially in developing countries. Nearly one third of the active epilepsy is due to NCC in the developing countries. A high proportion of patients with late-onset epilepsy, especially in endemic regions like Latin America, has been attributed to this aetiology[28,32]. In India many factors favourable for the transmission of the disease exists like low socioeconomic status, poor hygienic practices , pig rearing. But there is very little data on the prevalence of the disease in the general population or among epilepsy patients. The diagnosis of this disease is a major challenge as it presents with non specific clinical manifestations. Hence for the diagnosis and treatment of epilepsy due to NCC , knowledge of the prevalence of the disease in that region and a high index of suspicion is essential. Hence the present study was done with the objective to assess the seroprevalence and prevent the transmission of the *Taenia solium* in the population .

The population enrolled in this study included patients seeking medical attention and care for seizure or epilepsy , which included new onset seizure and chronic epilepsy patients, at Stanley hospital, Chennai. The control population included healthy individuals without complaints

of seizure or epilepsy . The 150 patients enrolled in the study were residents of Tamilnadu, coming from various areas in and around north Chennai. Detection of antibodies only in the serum was chosen , because lumbar puncture for the collection of CSF would be invasive and unethical unless indicated. Antibody detection in the serum by EITB was done both in the study and control population. We were able to perform ELISA only in the study group due to the unavailability of the kit at that time.

Age wise distribution of the epilepsy patients :

The study group included patients aged 13-64 years with the mean age of 28.42±12.76 years. In the present study epilepsy was seen in all age groups in the adult population , the majority of patients were in the age group of 21-30 years (33%) followed by <20 years age i.e 29% (Table1). In a study done by Goni et al [24](1962) majority of the cases were in the age group 20-40 years . A study done by Kuruvilla et al[58] (2001) at a tertiary care hospital in Kerala, reported age range of 24-35 years with mean age of 35.2 years. The age distribution of the epilepsy patients in the present study is similar to results of the above studies.

Gender distribution among epilepsy patients :

Of the 100 epileptic seizure patients studied , 64% were males and 36% were females with male to female ratio of 1.8:1 (Table2). Similar distribution was seen in a study by Parija et al [53](2011) in which males were 60.8% of the epileptic seizure patients studied.

Seizure pattern seen in the study population :

Humberto et al studied neurocysticercosis and epilepsy in Africa , and found that generalised seizures was reported slightly more often than partial seizures. Of the generalised seizures, tonic clonic type was the most often reported. In his study , among the patients with epilepsy , seizures within past year was seen in 28.9% and seizure for than 10 years was seen in 31.2% In our study, among the 100 epileptic patients about 41% presented with seizure duration of less than 1 year (new onset seizure). The majority of these patients belonged to < 20 years (Table3) . Chronic epilepsy constituted 59% of the study population with the highest number in the 21-30 years age group.The most common seizure type in this group was generalised tonic clonic seizure (GTCS) seen in 73% of the study population (table4). Partial type of seizure was seen in 27% of the patients. The results obtained in our study is similar to the above study.

Pork consumption and other potential risk factors among the study group :

In the present study , only 18% of the study group and 8% of the control group gave history of consumption of pork (table 5). This is less than that reported by Kuruvilla et al [37], who reported 36% of pork consumption among his study group . The low percentage of pork consuming population in the present study is due to the dietary habits of the people in this region. Nearly 45% of the cases in the study group reported absence of sanitary infrastructure. This results in open areas and field defecation. Free roaming pigs have access to these human feces and perpetuate the transmission of the parasite from pigs to humans.

MRI findings :

When MRI was used to identify cases , about 20% of the epileptic patients were diagnosed as NCC. (table 6). Nodular Calcificied inactive lesions were detected in only 2% of the cases in the present study. However colloidal vesicular stage presenting as granuloma was the most common lesion in MRI identified constituting 11% among the epileptics , 55% of the suspected NCC cases. Similar results were shown in a meta analysis by

Rajasekhar et al [54] , who reported that 60-70% of NCC cases in India present as solitary cystic granuloma. Mohanty et al[55] studied NCC prevalence in a north Indian hospital showed solitary granuloma to be the common radiological finding seen in 74.2% . . Of the radiologically suspected cases , nearly 60% of the cases were less than 30 years. Kotokey et al has reported that maximum incidence of neurocysticercosis in 21-30 years , seizures being the common presentation. The findings of our study correlates with findings of the above study.

Stool examination :

Of the 100 cases in the study group , stool sample was obtained from 48 cases . Taenia egg was not detected in any of these specimens tested. This may be because of the small number of specimens examined. A similar study done by Ashish et al[4] demonstrated Taenia eggs in 21.2% of the patients with multiple CNS lesion and 9.1% with solitary lesion in CNS. Similar study in the future with larger number of cases included might throw light on the prevalence of intestinal taeniasis .

Antibody detection by ELISA in epilepsy patients:

The diagnosis of neurocysticercosis is difficult as the sensitivity and specificity of the test depends on the clinical stage of the disease. Imaging techniques like CT and MRI provide quicker results but not available in low income settings. MRI has the advantage of detection of the various stages of the parasite. However it is expensive and not available in many hospitals. Serological tests available for antibody detection include ELISA and EITB.

ELISA has been shown to have variable sensitivities by various authors. It has added disadvantage of significant cross reactions with other cestode infections like hydatidosis[71,72] . In the present study, anti-cysticercal antibodies were detected in 25% of patients with epilepsy by ELISA (Table 8). Parija et al[24] who has done a similar study detected antibodies in 17.45% of the epilepsy population.In our study, antibodies to cysticercus of *T. solium* detected by ELISA was more in the age group of 21-30 years and majority presented with GTCS (table 8). Of the 23 seropositives, 11 were males (47.8%) and 12 females (56.5%) .No difference in the seropostivity between the two sex was identified. The above mentioned study done by Parija et al [24] reported that ELISA was positive in 56.44% of males and 43.56% of the females in the study population showing more seropositivity in males than females.

Results of EITB :

Seropositivity among the study population :

The study population was tested for anti-cysticercal antibodies by ELISA and EITB.EITB being the confirmatory serological test for cysticercosis, the seroprevalence was estimated based on its results. In the present study seropositivity among the epilepsy patients for IgG anti-cysticercal antibodies was 27%. This should be interpreted with caution as the study population was patients volunteering for medical care and not randomly selected. Hence at the community level the seropositivity may be still higher.

Other similar studies were,

Author	% Prevalence
Present study	27%
Ashish kumar et al[4]	32%
Kishore L et al [34] , 2004	35.9%
Kashi N Prasad et al (2009)[31] ,Lucknow north India	48.3%
Parija et al[24], Pondicherry(2010)	16.2%
V Rajasekar et al[43], Vellore district, India(2006)	13%

Basem et al ; (2010)	6.5%
L. Guillermo Palacio[42] et al, Columbia (1998)	9.82%
Mittal V et al[56] ,India (2001)	10.1%
Oscar H. Del Brutto[61] et al, Ecuador, 2004	8.6%

The seroprevalence obtained in our study is higher than previous studies done in South India. The high seroprevalence in this region may be due to the fact that most of the cases included were from areas in and out of North Chennai. Majority of the people living in this region , are below poverty line with low socioeconomic status , low sanitation and poor hygiene. Cysticercosis , said to be the `biological marker' of social and economic status of a region, well relates to the high seroprevalence in this region.

EITB is more specific than ELISA as the latter uses crude antigen for antibody detection . Whereas in EITB the crude antigen is seperated to the various glycoproteins which is then blotted on to a membrane. The antibody reacting to these specific antigens appears as bands.

In our study , antibodies to T.solium was detected in 27% of the epileptic population (table 9). Studies done in the various parts of the country have reported various seroprevalence rates. A community study

done at rural Honduras by Sanchez L et al [62] showed seroprevalence of 17%. A similar study done by Parija et al[53] reported anti-cysticercus antibodies in 16.2% of the epileptic patients studied. Mohanty et al reported anti-cysticercus antibodies in 14.1% of the neurocysticercosis in north India[44] . Seroprevalence of 35.9% was reported in Aurangabad by Kishore et al [34] among clinically suspected and MRI proven cases of neurocysticercosis. Previous studies conducted at Chandigarh [33], antibodies were detected in 17.4% to 29.2% of the epileptic patients.. In 1998, Ahuja et al [6]demonstrated antibodies in nearly 30% of the epileptic patients. Similar studies done in the epileptics around the various parts of the world have shown prevalence rates of 12% in Peru[26] (1993) , 44.6% in Cameroon[2], 10.8% in Morelos, Mexico [65].

Among the healthy controls tested , antibodies were detected in 6%. A similar study done at Vellore[62], South India , showed the seroprevalence of antibodies to *T.solium* in the seizure free study groups to be 15.9% .

In our study, the reactive samples showed antigen –antibody reactivity at the 40kDa and 24Kda band regions. This is similar to the bands patterns obtained by Parija et al who showed reactivity at 40kDa, 32kDa, 24kDa , 18 kDa regions [53] and Bucardo et al who found reactive bands frequently in 50kDa, 42-39kDa , 24kDa and 14 kDa regions.

Analysis of the seropositives by EITB (table 10):

Age : In this study , antibodies to *T.solium* was detected in all age groups , but the major distribution was found between 21-30 years. Community based studies like S arti et al have demonstrated increasing trend of seropositivity with increasing age peaking at 46-55 years. Such pattern of seropositivity was not detected in the present study as the study population had more cases < 30 years than >40 years.

Sex : Antibodies were detected slightly more in females (55.6%) than males (44.4%) but it was not statistically significant ($p > 0.05$). Most of the previous studies[24,4] have showed no significant association between seropositivity and gender which is similar to the findings of the present study.

Seizure type : Generalised seizure was the most common type of seizure observed. Ashish kumar et al [4] and Bucardo et al have also reported that generalised seizures is the most common seizure type seen in NCC cases.

Association of potential risk factors with the seropositivity:

About 72.2% of the pork eating population were detected of anti-cysticercal antibodies in the serum .In a study done by Blocher J et al[7] showed significant association with pork consumption and risk for NCC

. In this context it is to be stressed that cysticercosis is a feco-oral infestation and pork consumption is not a prerequisite. The high proportion of seropositivity in the pork consuming population indicates exposure to the T.solium eggs. This could be due to the autoinfection of the eggs from these cases by feco oral transmission or reverse peristalsis[71]. Pork consumption may be indirectly associated with exposure to the parasite, due to the presence of family members consuming pork , who may be tape worm carriers and may be a potential risk factor for the disease .

Comparison of the imaging and serology results :

Of the 20 radiologically suspected cases of NCC , antibodies to *T.solium* was detected by EITB in only 10 of them and the rest non reactive .(table 12). This can be because of the misdiagnosis by MRI of some cases and failure to differentiate from other differential diagnosis with similar radiological finding like tuberculoma. It was observed in the present study that only 29.9% of the cases reactive by EITB had MRI lesions suspective of NCC (table . 10)In about 17(21.3%) cases, though imaging showed no abnormality , antibodies were detected.This can be due to EITB detecting antibodies in patients with cysticerci in parts other than CNS also and the antibodies persisting after the disease is cured . This proportion is slightly lower than the 27%

detected by EITB . Similar studies done by Mohanty et al [44] and Guillerma et al [38] showed a higher proportion of cases detected by imaging than serology. In these studies , CT was used as the imaging modality for diagnosis. This may be the reason for the lower detection of NCC cases by MRI in the present study . In the later stages of the development of the cysticerci in the brain, it undergoes calcification.Such parenchymal calcifications are better visualised in CT than MRI

Comparison of ELISA and EITB :

Of the 23 samples positive by ELISA, 17 samples were found reactive by EITB (table 13). Taking the EITB as the gold standard test , the sensitivity of ELISA was 61.5% and specificity of 90.4% (table 14). False positivity was seen in 6 cases (Table 13). Kotokey RK et al , 2006 demonstrated that ELISA had sensitivity and specificity of 82.6% and 100% in definitive NCC cases and 78.3% in CT suspected cases. In a similar study done by Parija et al[53] at JIPMER , Puducherry ELISA showed low sensitivity of 91% and high specificity of 98%. Palacio et al[52] (1996) who studied the prevalence of NCC in epilepsy patients, showed ELISA had a sensitivity and specificity of 27% and 100% respectively. The above studies show that ELISA has variable sensitivity and specificity. False positives were detected in 6 cases. In our study

ELISA was found to have low sensitivity but high specificity. The low sensitivity of ELISA is due to the crude antigen used for the detection of antibodies. The complex conformation of these antigens stays inaccessible to the antibodies [48].The high false positives is due to the cross reactions with other infections like hydatid disease. Hence a positive result needs further confirmation with EITB. Western blotting though reported with high sensitivity and specificity has disadvantages. It is costly , requires expensive equipments, time consuming and hence it as not available in most of the health care settings.On the other hand, ELISA is rapid, easy to perform, cost effective and many samples can be processed . Hence ELISA can be used in community studies to assess the seroprevalence .

Proportion NCC cases causing active epilepsy:

The serological tests detecting for the presence of antibodies to *T.solium* can only assess the exposure to the parasite and its transmission in the community. Presence of these antibodies indicate the past or current exposure / infection to *T.solium* but not the presence of the disease . The lesions of NCC in imaging techniques like CT or MRI needs to be differentiated from other etiologies especially tuberculoma.

Hence the diagnosis of the disease status of the individual requires both imaging and serology findings .

In the present study, based on the modified Del Brutto's criteria diagnosis of neurocysticercosis was made in 8(8%) cases- 4 definite and 4 probable cases. (table 15)

Definite : major criteria – a) lesions suggestive of NCC in MRI and

b) positive serum EITB

and

minor criteria – Suggestive clinical feature (epileptic patients)

and

epidemiological criteria- patients living in highly endemic areas

Probable : 1major criteria : positive serum EITB

and

2 minor criteria: a) lesions compatible with NCC on MRI and

b) suggestive clinical feature (epilepsy)

In the present study the seroprevalence of the antibodies to T.solium was 27% by EITB and active NCC was seen in 8% of the epilepsy patient. Hence though there is high exposure to *T.solium* in this population , the proportion of active NCC cases causing epilepsy is lower.

SUMMARY :

Patients presenting with seizures or epilepsy , attending the neurology department as in-patients or out patients were enrolled and the study was conducted. All the cases were made to answer a questionnaire designed to assess the potential risk factors . After clinical and neurological evaluation , imaging by MRI was done in them . Serum samples were collected from 50 healthy individuals without complains of epilepsy as control population. Serum samples were collected and tested for the presence of anti-cysticercal antibodies to *T.solium* by ELISA and EITB.

In the present study, of the 100 cases following results were obtained

- Majority i.e 30% of the epilepsy cases were seen in the age group of 21-29 years and the males to female ratio was 1.8:1.
- Chronic epilepsy (> 1 year) was reported in 57% which is slightly more than that of incident seizures .
- Generalised seizure was the most common seizure type observed.
- Of the 100 epileptic patients studied, radiological diagnosis by MRI suspective of NCC was made in 20 cases.

- Colliodal vesicular stage as seen as granuloma was the most common lesion observed in MRI followed by ring enhancing lesions.
- Seroprevalence of anti cysticercal antibodies to *T.solium* was found to be 27% by EITB and in 6% of the control population.
- Majority of the seropositives were in the age group of 21-30 years , females slighltly more than males.
- Generalised seizure was the most common type observed among the seropositives.
- Antibodies were detected more in the new onset seizure group than that seen in the chronic epileptic patients.
- Consumption of pork was seen in 72.2% of the cases with anti-cysticercal antibodies
- Antibodies were detected in only 25% of the cases by ELISA.
- Taking EITB as the standard test, the sensitivity and specificity of ELISA was found to be 61.5% and 90.4% respectively . False positivity was seen in 30.4% of the cases.
- On comparing imaging and the serology findings , anti-cysticercal antibodies were detected in 50% of the radiologically suspected

NCC cases. Antibodies were present in 21.3% of the cases with normal imaging study

- Based on the modified Del H Brutto's criteria , definite and probable diagnosis of NCC was made in 4 and 4 cases respectively.

CONCLUSION :

Epilepsy is an important public health problem in developing countries like our country with the significant impact in the economy of the country . Neurocysticercosis is an important cause of acquired epilepsy contributing to nearly one third of the epileptic cases in developing countries. From the present study we conclude that the there is high level of antibodies to cysticercus of *Taenia solium* . Hence indicating the high level of exposure to *Taenia solium* in the epileptic population studied . Though there is high level of exposure to the parasite, the proportion of active disease due to NCC was lower in the epilepsy patients studied. Epilepsy is the predominant manifestation of NCC in our subcontinent. The study also shows that serological tests like ELISA and EITB aid in the better identification of clinically and radiologically suspected active NCC cases. ELISA has low sensitivity and high specificity than EITB in detecting the antibodies. But the lower cost and rapid results obtained makes it the serological test of choice in developing countries . The positive results requiring further confirmation with EITB. Overall the results of this study shows that there is rising trend of NCC and its seroprevalence in the epileptic patients. Hence NCC is a growing public health problem in our country and there is need to control this disease by proper sanitation and hygiene .

APPENDIX I:

Reagents required for ELISA :

- Microtitre plates coated with T.solium antigen
- Sample dilution buffer
- Wash buffer,Phosphate buffered saline (NaCl) solution (10 fold concemtrate)
- Positive control (diluted positive rabbit serum)
- Conjugate – Protein A conjugated with peroxidise
- Substrate : tetra methyl benzidine(TMB)
- Stop solution : 0.75M phosphoric acid
- Distilled /deionised water
- Distilled water
- Test tubes
- Disposable pipettes
- Vortex mixer
- Micropipette for 50-100µl and 1ml volume
- Stop clock
- Washing unit for microtitre plates or multi channel pippetes
- Photometer for microwell plates (450nm and reference filter ≥ 600nm)
- Filter paper(laboratory towels)
- Waste container containing 0.5% hypochlorite solution

Appendix-II:

REAGENTS REQUIRED FOR SDS PAGE ELECTROPHORESIS :

Working solutions

1.Acrylamide stock (30%)-100ml

a.Acrylamide – 29.2g

b.Bisacrylamide – 0.8g

c. Add distilled water to make 100ml and stir till completely dissolved

2.Seperating gel Buffer (4x)-100ml

a.75ml 2M Tris-Hcl(pH 8.8) - 0.5M

b.4ml 10% SDS - 0.4%

c.21ml of distilled water

3. Stacking gel buffer (4x) - 100ml

a. 50l 1M Tris-Hcl (pH 6.8) - 0.5M

b. 4ml 10% SDS - 0.4%

c. 46ml of distilled water

4. 10% Ammonium per sulphate (APS) -5ml

a. 0.5g APS dissolved in 5ml of distilled water

5.Electrophoresis / Running Buffer (1x) – 1000ml

a. 3g Tris - 25mM

b.18.8g Glycine - 250mM

c.1g SDS - 0.1%

d. Distilled water to make it 1000ml

6. Sample buffer -10ml

a.0.6ml 1M Tris – Hcl (pH6.8) - 60mM

b. 5ml of 50% glycerol - 25%

c. 2ml of 10% SDS - 2%

d. 0.5ml 2-mercaptoethanol - 14.4mM

e.1ml 1% Bromophenol blue - 0.1%

f. 0.9ml of distilled water

7. Staining solution – 1000ml

a. 1.0g Coomassie Blue R-250

b. 450ml of methanol

c. 450ml Distilled water

d. 100ml of Glacial acetic acid

8. Destaining solution - 1000ml

a. 100ml Methanol

b. 100ml of Glacial acetic acid

c. 800ml of distilled water

PREPARATION OF 10% SEPERATING AND 5% STACKING GEL :

FOR 10% SEPERATING GEL		FOR 5% STACKING GEL	
Acrylamide stack	3.3ml	Acrylamide stack	1.6ml
Separating gel buffer	2.5ml	Stacking gel buffer	2.5ml
Distilled water	4.2ml	Distilled water	5.9ml
10%APS	50µl	10%APS	50 µl

TEMED	20 µl	TEMED	20 µl
Total volume	10ml	Total volume	10ml

APPENDIX III

REAGENTS FOR WESTERN BLOT :

i) Transfer buffer

Tris 3.0g

Glycine 4.5g

Methanol 200ml

Distilled water make upto 1000ml

(pH between 8.1-8.4 without adjustment)

ii) Phosphate buffered saline (pH 7.2)

Na_2HPO_4 5.76 gm

$Na_2H_2PO_4$ 1.5gn

NaCl 9.0gm

Make the volume to 1000ml

iii) Blocking solution

3% BSA in PBS(7.2)

iv) Substrate buffer (PBS-T)

PBS (7.2) containing 0.1% Tween -20

v) Substrate solution for Horse Radish peroxidise

20% H_2O_2 10 µl

Substrate buffer 10ml

Diaminobenzidine (DAB) 6mg

vi) Antibody / Conjugate dilution buffer

0.55 BSA in PBS-T

vii) Washing buffer (PBS-T)

PBS (7.2) containing 0.1% Tween – 20 (1ml of tween -20 in 1litre of PBS)

DESTAINING SOLUTION :

1.25% Isopropyl alcohol

2. 10% Glacial acetic acid

3. Distilled water

Ponceau's stain :

1. 2% ponceau stain powder
2. 30% Trichloro acetic acid
3. 30% Sulfosalicilic acid

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PROFORMA

A. PATIENT DETAILS

1.Name :

2. Patient ID no :
3. Neurology no :
4. Age
5. Sex
6. Residence
7. Contact no
8. Religion
9. Occupation
10. Socio-economic status

B. HISTORY

1.Presenting Illness

1. Seizure :
    - Age of onset of seizures
    - New onset / chronic epileptic
    - Type of seizure – generalised / partial
    - Frequency of seizure

2Associated symptoms – headache , vomiting

3. Known case of tuberculosis

2..Past History :

- h/o contact with case of TB
- h/o trauma

3.Personal History :

- h/o consumption of pork
- toilet facilities
- h/o passing worms in the stool

- pig rearing
- any pet dogs

4. **Family history** :
   - Family h/o seizures
   - Known case of NCC among the family members
5. **Treatment History** :
   - Anti epileptic treatment
   - Anti helminthic drugs
6. **Travel History** :

   h/o travel to endemic countries

C. **CLINICAL EXAMINATION** :

1. **General examination**

   Presence of subcutaneous nodules

2. **Systemic examination**
   - Central nervous system
   - Cardiovascular system
   - Respiratory system

D. CLINICAL DIAGNOSIS

E. INVESTIGATIONS

a. CT findings

b. MRI findings

Normal study

Lesions suggestive of NCC

- Granuloma / calcification/ ring enhancing lesion with central scolex

F. Stool Microscopy:

G. Serology

1. ELISA : positive / negative
2. EITB : reactive / non reactive

S.No	H.No	Name	N.no	Age	Sex	Residence	new onse	chronic	duration	seizure type	headache	pork eating	toilet faci	AEDs	stool mic	MRI	ELISA	EITB	del brutto
1	78233	selvam	3452/12	27	M	red hills	+	-	10d	CPS	no	no	yes	no	-	normal study	negative	non reactive	
2	8134	rajakumari	7345/12	40	F	royapuram	-	+	30 yrs	GTCS	no	no	yes	irregular	-	normal	negative	non reactive	
3	78809	ambika	6961/12	15	F	pudhukuppam	+	-	4 months	GTCS	no	no	no	no	NA	normal	negative	non reactive	
4	78229	Geetha	1178/12	25	F	vILLUPURAM	-	+	3 mons	GTCS	no	yes	no	yes	NA	normal	negative	non reactive	
5	8625	kauveri	6840/200	30	F	Royapuram	-	+	12 yrs	CPS	no	no	yes	yes	-	normal	negative	non reactive	
6	8592	Dillibabu	1602/12	15	M	korukkupet	-	+	15 yrs	GTCS	yes	no	no	yes	NA	Ring enhancing lesion	negative	non reactive	
7	76630	nandha kumar	9007/12	42	M	Muthiramerrur	+	-	5 mon	GTCS	no	yes	no	no	-	granuloma	negative	reactive*	definitive
8	9639	devinandhini	7823/12	24	F	red hills	+	-	4mon	gtcs	yes	no	yes	no	-	old granulomatous lesion in R parietal regio	negative	non reactive	
9	78681	Sivakumar	1184/12	17	M	vILLUPURAM	+	-	2 mon	CPS	no	no	yes	no	NA	granuloma brain ?NCC	**positive**	**non reactive**	
10	79654	jayakumar	3456/10	45	m	minjur	-	+	5mon	GTCS	no	no	yes	no	NA	normal	negative	non reactive	
11	9647	Ganesan	2523/4	14	m	koodyanur	-	+	13 yrs	GTCS	no	no	yes	yes	NA	normal	negative	non reactive	
12	72343	vidhya	5645/12	27	F	tondiarpet	-	+	6y	GTCS	no	no	yes	yes	-	normal	negative	non reactive	
13	77689	rahman	5344/12	35	m	washerman pet	-	+	2 yrs	GTCS	no	no	no	yes	NA	normal	negative	non reactive	
14	78559	selvi	6734/12	28	F	thiruvallur	+	-	12 mon	CPS	no	no	no	yes	-	normal	**positive**	**reactive**	
15	78504	eswari	8963/09	21	F	korukkupet	+	+	16 y	CPS	yes	no	yes	no	-	acute granuloma L frontal gyrus ?ncc	**positive**	**reactive**	probable
16	78553	selvi	7823/12	39	F	parrys	-	+	20 y	CPS	no	no	yes	yes	NA	normal	negative	**non reactive**	
17	78567	vijay	8123/12	28	M	washerman pet	+	-	20 days	GTCS	no	no	no	no	-	normal	**positive**	**reactive**	
18	78738	marudhamalai	1194/02	20	m	thiruvallur	-	+	13 yrs	focal seizure	no	no	yes	no	NA	L frontal granuloma	**negative**	**non reactive**	
19	78934	thairu nisha	2312/08	37	M	washerman pet	-	+	6 yrs	GTCS	no	no	yes	yes	NA	normal	**negative**	**non reactive**	
20	78128	ramamoorthy	8233/12	48	M	nettum	-	+	20y	GTCS	no	no	yes	yes	-	normal	**positive**	**reactive**	
21	11589	santhosh kumar	7523/12	22	M	red hills	+	-	20d	GTCS	yes	no	yes	no	NA	normal	**negative**	**non reactive**	
22	12675	kannagi	8723/12	38	F	red hills	+	-	2 d	GTCS	yes	no	yes	no	-	normal	**negative**	**non reactive**	
23	12124	ravi	8905/12	25	F	p.kottai	+	-	3mon	GTCS	yes	yes	yes	no	-	normal	**negative**	**reactive**	
24	12658	Dilshan	4567/10	26	F	vyasarpadi	-	+	5 y	GTCS	yes	yes	yes	yes	-	ring enhancing lesion	**positive**	**reactive**	definite
25	14675	daksan	7623/12	43	m	korukkupet	+	-	2months	GTCS	yes	yes	yes	yes	NA	normal	**negative**	**non reactive**	
26	14672	udayabharathi	5635/2	15	F	P.nagar	+	-	4d	CPS	yes	no	yes	no	NA	normal	**negative**	**non reactive**	
27	14570	murugapandy	8742/12	22	M	washerman pet	-	+	7y	CPS	no	no	yas	no	NA	normal	**negative**	**non reactive**	
28	19648	ramkumar	1284/12	13	M	royapuram	+	-	2weeks	CPS	yes	no	yes	no	-	normal	**positive**	**non rective**	
29	11408	soundarapandiyan	5462/12	53	m	thiruvottiyur	+	-	10d	gtcs	no	no	yes	no	NA	multiple Ring Enhancing Lesions	**negative**	**non reactive**	
30	79806	muthu	3042/12	68	m	thiruvottiyur	-	+	1 y	GTCS	no	yes	no	no	NA	normal	**positive**	**reactive**	
31	79952	nalini	6152/12	42	f	vyasarpadi	-	+	3 y	GTCS	no	yes	yes	no	-	normal	**negative**	**non reactive**	
32	20694	nandhini	3120/12	20	f	vyasarpadi	-	+	3 y	GTCS	no	no	no	yes	NA	normal	**negative**	**non reactive**	
33	21902	suriya	5609/10	14	m	parrys	+	-	3mon	GTCS	yes	no	no	no	-	normal	**negative**	**non reactive**	
34	79957	ajmer	6193/10	22	m	manali	-	+	3 y	GTCS	yes	no	no	no	-	normal	**positive**	**reactive**	
35	50162	rajavaran	5016/12	25	m	tondiarpet	+	-	5 d	CPS	no	yes	yes	no	NA	normal	**negative**	**non reactive**	
36	79884	tmilselvi	8845/12	28	f	thiruvottiyur	-	+	5y	GTCS	yes	no	no	no	NA	normal	**negative**	**non reactive**	
37	80290	divya	3402/12	17	f	thiruvallur	-	+	15 yrs	tcs	no	yes	yes	no	-	normal	**negative**	**non reactive**	
38	80296	lakshmi	2024/12	19	f	korukkupet	-	+	2 yrs	gtcs	yes	no	no	no	NA	normal	**negative**	**non reactive**	
39	80298	krishnamoorthy	3012/12	39	m	vyasarpadi	-	+	20y	cps	no	no	no	yes	NA	normal	**positive**	**reactive**	
40	80198	vani	1833/06	18	f	thiruvottiyur	-	+	4y	gtcs	no	no	yes	yes	-	normal	**negative**	**non reactive**	
41	80122	saral balmet	3492/12	54	f	mambakkam	+	-	2 d	gtcs	no	yes	no	no	-	multiple Ring Enhancing Lesions	**positive**	**non reactive**	
42	80345	arjunan	2077/`12	65	m	minjur	+	-	6 mon	cps	no	yes	yes	no	NA	normal	**negative**	**non reactive**	
43	80580	gunasekaran	5673/12	20	m	kalahasti	+	-	8mon	gtcs	yes	no	yes	no	NA	granuloma	**negative**	**non reactive**	
44	23046	pandiyan	3425/12	17	m	aaradi	-	+	2 yrs	gtcs	no	yes	no	no	-	granuloma L parietal region ?NCC	**negative**	**reactive**	probable
45	23021	suresh	8025/12	33	m	pulianthope	-	+	33y	gtcs	no	no	no	no	NA	normal	**negative**	**non reactive**	
46	23546	patchiyappan	3962/6	23	m	thiruvottiyur	_	+	18 y	cps	no	no	yes	no	NA	REL	**negative**	reactive	
47	24564	lakshmi	3927/12	26	f	bharathi nagar	-	+	3y	tcs	yes	no	yes	no	-	normal	**positive**	**reactive**	
48	24785	vadivel	5364/12	19	m	vILLUPURAM	-	+	15y	gtcs	no	no	no	yes	-	normal	**negative**	non reactive	
49	24165	ramesh	2453/2	24	m	tondiarpet	+	-	4d	CPS	no	no	no	no	NA	normal	**negative**	**reactive**	
50	25122	khDER MOIDEN	5628/12	44	m	washerman pet	-	+	15y	GTCS	no	no	yes	yes	NA	calcification	**negative**	non reactive	
51	81265	hemanth kumarr	8845/2	13	m	washerman pet	-	+	1y	cps	no	no	no	no	NA	normal	**negative**	non reactive	
52	25456	nagaraj	7834/11	13	m	tondiarpet	-	+	6y	GTCS	no	no	yes	yes	-	normal	**negative**	reactive	
53	26346	priyadharshini	5432/12	13	f	tondiarpet	+	-	10 d	GTCS	no	yes	yes	no	-	REL	**negative**	non reactive	

54	26430	vanitha begum	5645/11	17	f	washerman pet	-	+	12 yrs	GTCS	no	no	no	yes	NA	normal	**positive**	non reactive	
55	26897	suresh kumar	7865/6	24	f	velllore	-	+	14y	GTCS	no	no	yes	yes	NA	normal	**negative**	non reactive	
56	27675	saravanan	3435/12	15	m	kaasimedu	-	+	10y	GTCS	no	no	no	yes	-	normal	**negative**	non reactive	
57	27756	devi	5546/2	27	f	vyasarpadi	+	-	7 d	GTCS	no	no	yes	no	NA	normal	**negative**	non reactive	
58	27933	baby shalini	5673/12	13	f	red hills	+	-	3 mons	GTCS	no	no	yes	no	NA	normal	**negative**	non reactive	
59	81234	koomathi	6342/12	25	f	pulianthope	-	+	5 yrs	GTCS	yes	no	yes	no	-	normal	**positive**	mildly reactive	
60	81212	kamesh	4599/12	32	m	bharathi nagar	+	-	1week	GTCS	yes	no	yes	no	NA	normal	**positive**	reactive	
61	28190	praKASH	6123/2	30	M	vyasarpadi	-	+	6MON	GTCS	yes	yes	yes	no	NA	normal	**positive**	non reactive	
62	28234	raziya sultan	6199/12	23	f	koodyanur	-	+	1 year	GTCS	yes	no	yes	yes	-	normal	**negative**	non reactive	
63	81267	devi	6178/2	17	f	korukkupet	-	+	2y	CPS	no	no	yes	yes	NA	normal	**negative**	non reactive	
64	28111	mohan	5633/12	50	m	royapuram	-	+	2y	GTCS	no	yes	yes	yes	NA	granuloma	**positive**	reactive	probable
65	28746	adiyamma	6477/3	35	f	A.bakkam	-	+	10yrs	GTCS	yes	no	yes	yes	NA	granuloma	**negative**	non reactive	
66	81243	kanagaraj	4318/12	21	m	washerman pet	+	-	2y	CPS	yes	yes	no	no	NA	normal	**negative**	non reactive	
67	81376	anandhi	6529/12	19	f	tondiarpet	+	-	5 y	GTCS	yes	no	yes	no	NA	normal	**positive**	**reactive**	
68	29236	vedachalam	6734/12	32	m	arani	-	+	3yrs	GTCS	yes	no	yes	yes	-	normal	**negative**	non reactive	
69	29234	vankatesan	7623/12	34	m	r.hills	+	-	2 weeks	GTCS	yes	no	yes	no	NA	normal	**negative**	non reactive	
70	29432	suriyan	6799/12	30	m	Sharma nagar	+	-	7 d	GTCS	no	no	yes	no	NA	normal	**negative**	non reactive	
71	81379	Aruna	6783/12	48	m	arani	-	+	1 yr	GTCS	no	no	yes	yes	NA	R parietal speckle of calcification	**negative**	non reactive	
72	81397	shekar	7012/12	14	m	p.palayam	-	+	9yr	GTCS	no	no	yes	yes	-	normal	**negative**	non reactive	
73	81423	padmavaty	7019/12	22	f	korukkupet	+	-	4 d	GTCS	no	no	yes	yes	NA	normal	**positive**	non reactive	
74	20310	selvaraj	7123/12	55	m	p.thope	+	-	3d	GTCS	no	no	yes	no	-	normal	**negative**	non reactive	
75	81333	soundarya	7130/12	15	f	ponneri	+	-	11mon	CPS	no	no	no	no	-	normal	**negative**	**positive**	
76	29345	geetha	7590/12	24	f	mayavaram	+	-	2months	GTCS	no	yes	yes	no	NA	normal	**positive**	non reactive	
77	29223	manjula	7623/12	35	f	vilLUPURAM	-	+	4 y	CPS	no	no	yes	yes	NA	normal	**negative**	non reactive	
78	29241	gubendran	7699/12	15	m	vILLUPURAM	+	-	3d	CPS	no	no	no	yes	-	ring enhancing lesion(acute granuloma in L	**positive**	**positive**	probable
79	81629	udhayakumar	4146/12	48	m	washerman pet	-	+	6 minths	CPS	yes	no	yes	yes	NA	granuloma	**negative**	non reactive	
80	29384	vasanti	3212/12	20	f	washerman pet		+	1 1/2 y	CPS	yn	yes	yes	yes	-	normal	**negative**	non reactive	
81	81656	geetha	2145/12	31	f	minjur	-	+	4y	GTCS	no	no	yes	yes	NA	normal	**positive**	**reactive**	
82	81690	malliga	3490/12	45	f	k.nagar	+	-	2d	GTCS	yes	no	yes	yes	-	normal	**negative**	reactive	
83	81634	lokesh	6623/12	15	f	ponneri	+	-	5d	CPS	yes	no	yes	yes	-	normal	**negative**	non reactive	
84	81678	suresh	4329/12	25	f	mandaveli	-	+	12 yrs	GTCS	no	no	yes	yes	-	granuloma	**positive**	reactive	probable
85	81645	pavithran	4962/12	13	f	r.hills	+	-	20 days	CPS	no	no	yes	yes	NA	normal	**negative**	reactive	
86	81578	raja	5432/10	17	m	senji	-	+	2y	GTCS	no	no	yes	yes	NA	normal	**negative**	non reactive	
87	81690	thilakavathy	5674/12	17	f	nettum	+	-	6mon	GTCS	yes	no	no	no	NA	normal	**negative**	non reactive	
88	81703	gopinath	7866/12	33	m	koodyanur	-	+	13 yrs	CPS	no	no	no	yes	-	normal	**negative**	non reactive	
89	81754	amudha	3487/12	22	f	korukkupet	-	+	1 1/2 yrs	GTCS	no	no	yes	yes	-	normal	**negative**	non reactive	
90	81796	abdul kathar	4577/12	25	m	washerman pet	+	-	6mon	GTCS	yes	no	no	no	-	normal	**negative**	reaactive	
91	81245	shankaran	6723/12	16	m	red hills	+	-	3 wks	GTCS	yes	no	yes	no	NA	normal	**negative**	non reactive	
92	81823	divya	4783/12	26	f	tondiarpet	+	-	3mon	GTCS	no	no	yes	no	-	normal	**negative**	non reactive	
93	81817	mannar	4352/12	34	m	royapuram	-	+	11 yrs	GTCS	no	no	yes	yes	-	normal	**negative**	non reactive	
94	81745	devi	5634/12	45	f	bharathi nagar	+	-	4mon	GTCS	no	no	yes	no	NA	normal	**negative**	non reactive	
95	81913	jenifer	7011/12	55	f	royapuram	-	+	14 yrs	CPS	yes	no	yes	yes	-	normal	**negative**	non reactive	
96	81934	jagan	5673/12	19	m	parrys	+	-	1 d	GTCS	y	no	yes	no	NA	normal	**negative**	non reactive	
97	81967	eswari	4523/12	23	f	tondiarpet	-	+	3 yrs	GTCS	no	no	no	yes	-	normal	**negative**	non reactive	
98	81924	esther	7865/6	39	f	minjur	-	+	5 yrs	CPS	yes	yes	yes	no	NA	normal	**negative**	reactive	
99	81922	begum	4537/12	48	f	korukkupet	-	+	10 yrs	GTCS	no	no	yes	no	-	normal	**negative**	non reactie	
100	81945	muthu	5648/12	50	m	korukkupet	-	+	8 yrs	GTCS	no	no	yes	no	-	normal	**negative**	non reactive	

S.No	H.No	Age	Sex	pork consumption		EITB
1	324356	34	m	no	-	non reactive
2	45231	28	m	no	-	non reactive
3	23411	23	m	no	-	non reactive
4	24673	15	m	no	-	non reactive
5	23434	45	m	no	-	non reactive
6	23455	19	m	no	-	non reactive
7	32411	29	m	yes	-	non reactive
8	32412	45	f	no	-	non reactive
9	33210	27	m	no	-	reactive
10	32567	25	f	no	-	non reactive
11	32674	17	m	yes	-	non reactive
12	32145	44	m	no	-	non reactive
13	33782	40	m	no	-	non reactive
14	33546	25	m	no	-	non reactive
15	33780	29	f	no	-	non reactive
16	34678	19	m	no	-	non reactive
17	34526	18	m	no	-	reactive
18	34522	32	f	no	-	non reactive
19	34590	20	m	no	-	non reactive
20	37689	32	m	yes	-	non reactive
21	45232	30	f	no	-	non reactive
22	34253	28	m	no	-	non reactive
23	46523	46	m	no	-	non reactive
24	44537	16	f	no	-	non reactive
25	41786	40	f	no	-	non reactive

S.No	H.No	Age	Sex	pork consumption		EITB
26	49094	26	m	no	-	non reactive
27	48887	27	f	no	-	non reactive
28	47655	17	m	no	-	non reactive
29	343678	53	f	no	-	non reactive
30	34523	51	f	no	-	non reactive
31	35784	34	m	no	-	non reactive
32	23563	29	m	no	-	non reactive
33	45234	19	m	no	-	non reactive
34	46577	14	f	no	-	non reactive
35	45323	23	f	no	-	non reactive
36	45673	16	m	no	-	non reactive
37	45699	22	m	no	-	non reactive
38	63424	54	m	no	-	non reactive
39	45874	15	f	no	-	non reactive
40	45346	32	m	no	-	non reactive
41	56344	44	f	no	-	non reactive
42	26459	33	f	no	-	non reactive
43	24354	38	f	no	-	non reactive
44	45242	16	f	no	-	non reactive
45	56355	29	f	no	-	non reactive
46	56332	33	m	no	-	non reactive
47	56882	38	m	no	-	non reactive
48	48265	15	f	no	-	reactive
49	58732	26	f	no	-	non reactive
50	67454	60	m	no	-	non reactive

NA - NOT AVAILABLE
REL - RING ENHANCING LESION
M-MALE
F- FEMALE
GTCS- GENERALISED TONIC CLONIC SEIZURE
CPS - COMPLEX PARTIAL SEIZURE

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